Zeitschrift: Archives des sciences et compte rendu des séances de la Société
Herausgeber: Société de Physique et d'Histoire Naturelle de Genève
Band: 42 (1989)
Heft: 1: Archives des Sciences

Artikel: Polarity : from dipoles to biopolarization
Autor: Turian, Gilbert
DOI: https://doi.org/10.5169/seals-740080

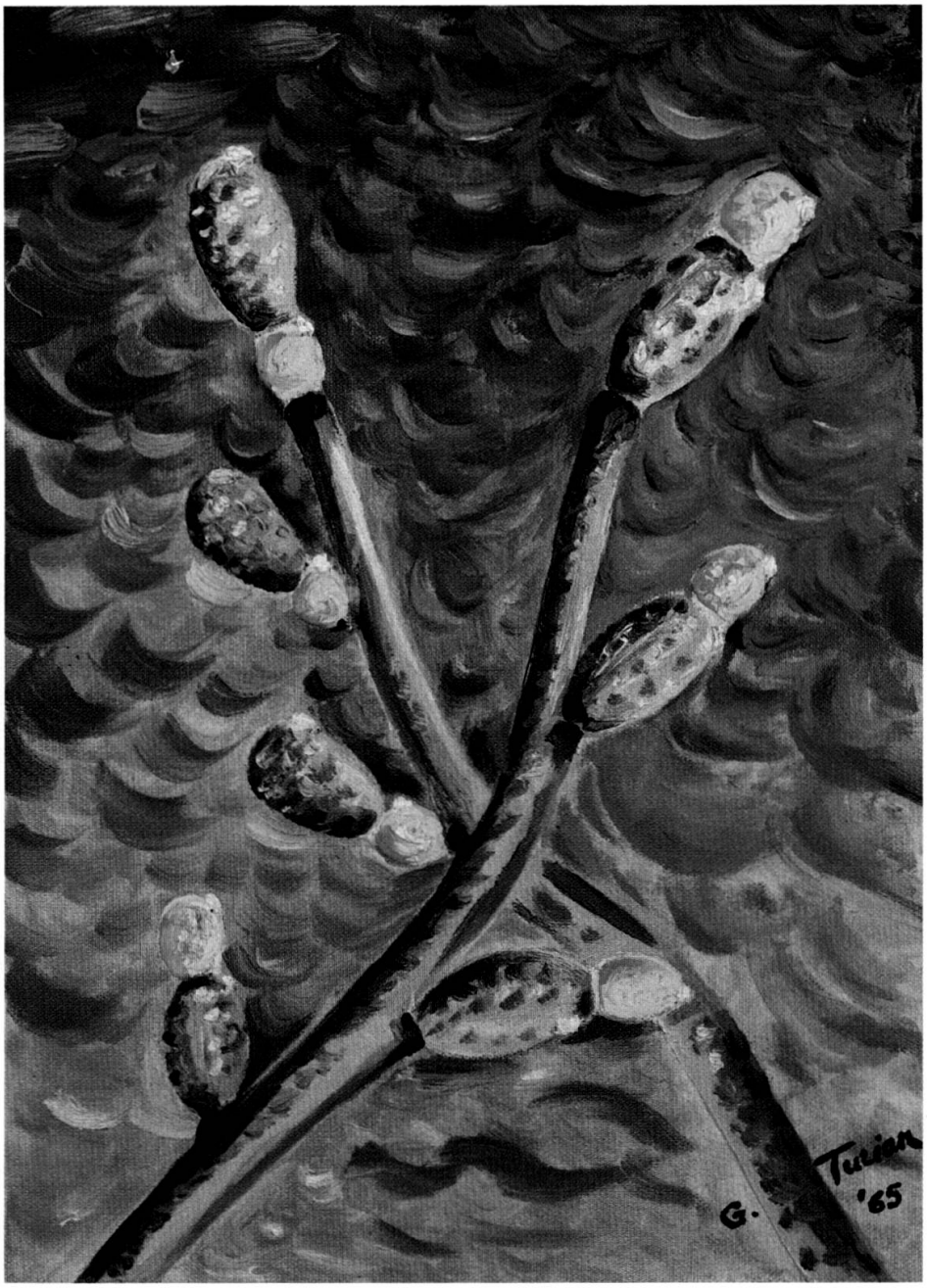
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DNA-positioned "sexual dipoles" (orange male gametangia) in the *Allomyces*.

POLARITY
FROM DIPOLES TO BIOPOLARIZATIONS

BY

Gilbert TURIAN *

PROLOGUE

Two decades ago, our sparkle to write "Différenciation fongique" (1969) was already a fascination for the polarized growth pattern of fungal hyphae. This decided us to follow them as our red tape to further focus our developmental interest on polarity alone, considered as unitarian principle pervading all natural processes, from inert to living matter.

Surprisingly, to our knowledge there was no book overviewing polarity in its broad span. This gap gave us the incentive to survey all possibly available data about polarity, from its physico-chemical fundaments to its more advanced biopatterns. However, in this attempt, we were soon confronted with the multitude of processes involving polarity and, therefore, had to be more selective than comprehensive in our choice of the models, sequentially presented from the primeval dipoles.

Microbial and plant models of biopolarity have been the easiest to select and present because of our direct involvement in these fields. However, we must ask for indulgence for the animal models, not to mention unavoidable omissions in the physical and chemical aspects of polarity.

In achieving this multifaceted task, we gratefully acknowledge the encouragements of Dr. Jacques Deferne, redactor of the *Archives des Sciences,* the steady secretarial assistance of Ariane Fehr, the fruitful discussions with my collaborators and colleagues of the different fields, and ... the patience of my wife and children, Jacques and Sandrine, and of all my friends.

We are also grateful to the *Société Académique de Genève* for partial financial help.

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INTRODUCTION

Polarity can be currently defined as two different qualities opposed at the extremities of an axis or, more simply, as axial asymmetry generating polar axiation. In this connotation, it means heterobipolarity, namely two different poles on an axis as examplified by the dipolar magnetic bar with its north and south poles; contrarily to theoretically impossible homobipolar magnets, monopoles of the north or south type are predictable as homologues of the isolated + or − electric charges. Polarity is thus fundamentally bipolar and originates from the lasting separation of opposite, elementary electric charges, the proton (H^+) and the electron (e^-) in the probabilistic, primeval dipole hydrogen (H). From that point of view, electric and magnetic bipolarities of Physics rejoin the dual principle of Yin-Yang proposed by oriental Philosophy, a problem of opposed polarities which could be solved by the modified notion of structural stability recently proposed by Zeeman (1988).

In Biology, the term polarity was introduced by Allman in 1864 in connection with the specific orientation of phenomena of animal regeneration with reference to axis in space and, in 1904, the great *Drosophila* geneticist T.H. Morgan called "polarity" the dominance of the apical parts in the axial regeneration of hydranths. In plants, the theoretical aspects of polarity have also been extensively discussed in their relation to problems of regeneration, directional growth and differentiation. In 1878, Vöchting showed that a stem or cutting is polarized. He attributed this property to polarity of individual cellular components and, ultimately, to their molecular structure. In that pioneer view of the molecular essence of polarity, he was followed, in 1935-36 only, by Seifriz, who considered the protoplasm as a continuity of structure consisting of elongated molecules (as in carbohydrates and proteins) to which the polarity and symmetry of organisms could be attributed. Harrison (1936) made the close proposal that protein molecules, because of their bipolar character, might become oriented in the cell possibly in relation to their point of attachment. In 1939, Paul Weiss had already hypothesized for animal eggs "the possibility of a *dynamic* structure, that is, a defined pattern into which the diverse molecular groups would force one another by mutual specific repulsions, attractions and other interactions". For Needham who had referred, in 1942, to "the difficult question of polarity, perhaps the central puzzle of embryonic development", constituent molecules of the cell protoplasm were also oriented in a definite structure which, in morphogenesis, was manifested as polarity. Bünning expressed again, in 1952, the opinion that the physical asymmetry induced within the young egg cell or spore must be a polar protoplasmic structure. According to him, the surface of protoplasm would therefore contain a submicroscopic protein skeleton exhibiting a polar orientation.

The structural views of biopolarity were first tempered by Child (1941) who saw the need for introducing metabolic factors without which the structure must remain static, if it can indeed come into existence. According to this eminent precursor, "molecular orientation must result secondarily from the metabolic differential pattern". Turing went further, in 1952, when he proposed his theory of the reaction-diffusion to account for the zygotes and eggs differentiation without invoking their specific protoplasmic structural organization. More recently, Prigogine (1972) attempted to reconcile cell submorphology and biophysics in considering membrane bound polarity, especially conspicuous in some types of cells, as the expression of dissipative processes far from thermodynamic equilibrium.

At the *single* cell level, the intrinsic polarity of macromolecules and their derived microstructures prevails over polarization by gradiential diffusion of morphogens (Grant, 1978). When transiently, apparently apolar spherical cells such as microbial spores or unfertilized eggs are led to recover their internal polarity, an intracellular gradient lines up polar molecules in specific orientation "like iron fillings in a magnetic field or opposite electrical charges at the two poles of an electrophoretic system". Founding this opinion on the pioneer bioelectrochemical studies of Lund (1947), Sinnott (1960) predicted that "polarity may be due to something quite different from a gradient and may be comparable to, and perhaps result from, an inherent polar tendency, presumably electrical in character". The intrinsic polar organization of cells should thus not only be based on the polarity of their macromolecules and microstructures but would also be tightly connected with their somehow hidden sensitivity to electrostatic and electromagnetic signals from the environment (Racusen, 1979; Berry, 1981; Pohl, 1981).

In the *multi*cellular organization of embryos, polarity reversal by grafting experiments provided strong evidence to Slack (1976) that "polarity does not result from many small polar substructures from the slope but of graded distribution of morphogenetic substances". On that experimental basis, Meinhardt theoretized, in 1982, that interactions and movements of molecules can also generate spatial differences or polar patterns from more or less homogeneous molecular conditions if two morphogenic substances with different diffusion rates react with each other by Turing's mechanisms. Such processes would provide positional information for the cells and would change their internal state of determination in a systematic way until it corresponds to the external signal, the local morphogen concentration. Morphogen polar gradients could thus direct the overall polarity of tissues up to organismic level. Nevertheless, the questions remain still largely unsolved of "what is the relationship between polarity and the origin of morphogenetic field? How does pattern formation occur within a morphogenetic field and, what does a position effect mean?" (Karp and Berrill, 1981).

From the above fundaments, we have conceived and surveyed a progressive, evolutive ladder of polarity upgraded through several levels of complexified integra-

tion: from the primary processes of electric bipolarization (chapter I) emerge small molecular dipoles, prototyped by H_2O, evolved into electrically mono — bipolar, often chiral (D/L-stereoisomers) biomolecules (chapter II); these polarities are repercuted into the macromolecules with the polarly-read sequences of nucleic acids and their translated proteins (chapter III); from these natural polymers emerge microstructures either bipolarly electrified as cell membranes or structurally polarized into head and tail as actin-myosin and tubulin, the components of microfilaments and microtubules respectively; such microstructures exhibit unidirectional growth during polymerization correlated with elongation of mitotic spindles or of surrounding cytoplasm (chapter IV); that cytoplasmic expansion is transient and polarly reversible in the cytoplasmic streaming of amoeboid pseudopods illustrating polar cell movements (chapter V) while it is permanent and axially continuous at polarized cell growth (chapter VI) and differentiation resulting from asymmetrical cell division (chapter VII); it is only at the ultimate organismic level of morphogenesis that intrinsic cell polarity becomes superceded by gradients of morphogens (chapter VIII).

Polarity can, however, not only be considered as an expression of the *spatial* component of development; at the genetic level, it is also thought to be regulated by *temporal* sequences of gene expression. In many cases, the translated products become localized within particular places in the cell such as the various wall specializations of plants (Sinnott, 1960). In our last chapters (V-VIII), we will therefore refer to the elusive problem of intracellular choreography (Fulton, 1984) by which such gene products are asymmetrically distributed at the onset of polarity (Schnepf, 1986).

I. ATOMIC POLARIZATIONS

A. ORIGINS

According to Einstein special theory of relativity, the "actors" of the fundamental duo matter — energy are interconvertible. Energy might have preceded matter and thus was there ever a time when no matter, of any sort, existed? Radiant energy could thus have preceded matter in the dualistic nature of the universe. However, and according to the widely accepted scenario (Weinberg, 1977), the radiations and matter were originally compressed in a sphere of fire; its diameter was minimal but its density and temperature were extremely high. At zero second of cosmological time, a gigantic cosmic explosion, the hot Big Bang first proposed in 1935 by the physicist Georg Gamow originated an uniform and still lasting isotropic expansion of the universe sequentially followed by the Planck time of particle creation.

In these first seconds of the universe, extremely energy rich photons (massless and chargeless weight packets of electromagnetic energy or quanta of light) would have given birth to the first elementary particles among which electrons (−) and positrons (+) forming the primeval, bipolar couple of elementary electric charges. Parallely, to these light leptons, also arose the + and − charged constituents of nuclear matter, the quarks, which associated into the heavier hadrons, proton and neutron, soon followed by the nuclei of the helium (2 protons + 2 neutrons). This radiation era of nucleosynthesis was preceded by an hadronic era of annihilation of proton-antiproton pairs and a leptonic era of annihilation of electron-positron pairs (Barrow and Silk, 1980). However, when temperature lowered, photons no longer were powerful enough to dissociate the atoms formed and every proton (H^+) became covered with an electron, forming the primordial atoms of hydrogen (H), every biprotonic nucleus of helium captured two electrons, etc. Electrically neutral atoms were thus born and the universe had passed its atomic phase.

The atomic binding between the proton and the electron is not saturated. Samples of atomic hydrogen gas could only be kept as such by exploiting the effect of spin polarization (Silvera and Walraven, 1982). The stabilized H atoms were produced by spin-polarizing all the electrons in the same direction (see I.D.4); instead of binding into pairs during a collision, they separate and H_2 will not form (Laloë and Freed, 1988). Otherwise atomic hydrogen is found strongly bonded to other elements to form chemical compound such as water, carbohydrates and other organic substances. Its ionization, when the electron escapes the electrical attraction of the proton, requires high energy as provided by electromagnetic (microwaves) radiation (quantum chaos? see Pool, 1989). Nevertheless, because of its feeble ionization in neutral H_2O (10^{-7}M H^+), the H atom provided the fundamental ion implicated in the biogenic redox reactions (see IV.B.2c).

Hydrogen as a gas made up of isolated atoms is not stable in the conditions that prevail on the surface of the earth and the atoms combine explosively to form the diatomic molecule of hydrogen (H_2) in which the two charged particles are maintained by electromagnetic forces. The two electrons can now circulate on complex orbitals around the two adjacent protons (McWeeney, 1986) and the hydrogen molecule formed is then a closed system with increased stability. The H-H molecule vibrates on a time scale of femto-second (10^{-12}s) and when a H atom approaches the H_2 molecule, to form a transition state (Eyring, 1936, see Zewail, 1988), the three hydrogens of the H_3 species transitorily formed "stick" together for about 10 fs (Zewail, 1988).

B. Symmetry - Polarity

In nuclear physics, the most basic information pertains to symmetry properties, and one of the first things any worker in the polarization field learns is the effect of parity and time reversal invariance on the relations between measurable polarization parameters, such as those between polarization and asymmetry (Barshall, 1975).

There is a long history of the study of symmetry principles in physical systems (Elliott and Dawber, 1979; Lee, 1988). In one of its aspects, it concerns the three dimensional rotational symmetries of atoms and molecules, and the translational and rotational symmetries of crystal lattices. Otherwise, the more abstract symmetries are associated with the internal structure of elementary particles and extend the familiar concept of electric charge (see I.C.1).

The principle of symmetry is satisfied when two different qualities are equivalent, as in the complementary "couple" matter/antimatter: charge symmetry implies that the antiworld with opposite charges is equivalent to ours (Weisskopf, 1969).

Under the combined inversion of charge and parity, the mirror image of any observed phenomenon represents the corresponding phenomenon, with each particle replaced by its corresponding antiparticle. This idea of antiparticle arose from Dirac (1929-30, see Davies 1980) who made the bold suggestion that perhaps there are really two types of matter: ordinary particles like electrons, and "mirror" particles of antimatter. To each particle should correspond an antiparticle endowed with an equal mass but an opposite electrical charge: the matter atom of H is formed of a positive proton plus a negative electron while the antimatter anti H is formed of a negative antiproton plus a positive antielectron or positron (von Egidy, 1987).

The level of particle-antiparticle asymmetry in the universe, which determines the entropy, is not absolutely invariant. It occurred after the early instants of the universe, when the decay processes of protons were abundant (Weinberg, 1981). A stable level of asymmetry between particles and antiparticles was eventually frozen and the predicted value became close to the observed asymmetry of one part in 10^8 (Barrow and Silk, 1980). Complex processes involved the non-conservation of the

symmetry of particles and anti-particles and generated a sharp unbalance between matter and anti-matter; they erased any memory of the initial entropy per nucleon (Georgi, 1981).

It is expected that an antiworld would look essentially like ours, but with opposite electric charges. If all properties of particles and antiparticles are completely symmetric the question therefore arises as to "why our world is made of particles and not of antiparticles, and what happened after the Big Bang, so that only matter survived ?" (von Egidy, 1987).

The fact that the so-called "weak force", the fourth of the basic forces in nature along with the strong, electromagnetic and gravitational forces, shows important left-hand — right-hand asymmetries (chirality, see II.D) could be an answer to the universe's option for a lopsided, matter-dominated arrangement.

C. Electric bipolarization

An external electric field can induce a slight relative shift of positive and negative electric charges in opposite directions within an insulator, or dielectric. In all atoms, electric polarization can be provoked by an electric field which distorts the negative cloud of electrons around positive nuclei in a direction opposite the field. The slight separation of charges makes one side of the atom somewhat positive and the opposite somewhat negative. The electric field has thus induced an electric dipole moment in the atom which can then be considered as polarizable.

1) *Electric charges*

According to the standard model of elementary processes, all matter is made up of hadrons — protons/neutrons — components (quarks) and leptons (electrons, neutrinos, muons, etc.), which interact with one another through four forces: gravity, electromagnetism, the weak force and the strong force. Recently, the still undiscovered Higgs boson has been introduced to make that standard model mathematically consistent (Veltman, 1986). Electromagnetism which is the best described of these four forces that govern the interactions of matter and energy accounts for the quantization of electric charge, i.e. that charge always comes in discrete multiples of a fundamental smallest charge (Georgi, 1981).

Interconversions can occur among hadrons: a neutron can become a proton (+), emitting an electron (−) called a β-particle, while a proton can also turn into a neutron, emitting a positron — a positively charged particle that can then combine with an electron to produce γ-rays.

As prototype of the leptons, the electron has a small mass and one unit of electric charge considered by convention as negative. Muons can also carry a negative electric charge and have properties quite similar to those of the electrons even though they are much more massive. Contrarily to the leptons, which are free particles, the quarks

are only constituents of heavier particles, the hadrons. In their allowed combinations the fractional electric charges of the quark add to yield an integral total charge. The *u* quarks have a charge of $+2/3$ while the charge of the *d* quarks is $-1/3$. The charged proton has the quark composition *uud* thus giving it a total electric charge of $+1$. That positive electric charge carried by the proton is equal in magnitude but opposite in sign to that of the 1,820 times lighter electron. The quarks in a proton or a neutron are thought to be held together by a new long-range fundamental force called the color force which acts on the quarks because they bear a new kind of charge called color (Harari, 1983).

Unionized atoms are electrically neutral because the negative charges on the electrons precisely balance the positive charges on the nuclear protons (Georgi, 1981). However, this deeply-rooted concept of balancing is worth a second thought: why should electrons and protons which seem to be independent forms of matter know so precisely the magnitude of each other's electrical charge?

An answer that has suggested itself to many is that ultimately there is but one type of matter: electrons and protons are siblings. If so, this would be the culmination of a long search for an underlying unity in nature. This startling result is a consequence of the electrical charge equality between the electrons and protons. But how sure are we that these charges are identical, and what consequences would ensue if they were different? Einstein had already suggested that the electron and proton charges (Q_e, Q_p) might not be precisely equated. In electrodynamics, and in the electroweak theory of Glasgow-Salam-Weinberg, the electric charges are even not fixed: proton and electron could have arbitrary charges. The reason for this would lie in the mathematical group structure of the theories. Moreover, in the description of electromagnetic interactions the much searched grand unification theory would not be ruled out by a difference in electron and proton charges, albeit small (Okun *et al.*, 1984).

However, the quantization of electric charge (particle's charges are integral multiples of the proton's charge) implies the exact neutrality of the atom (see above). This means that the strength of any given electromagnetic interaction is dependent on the size of the participating charges. Its measure is the so-called electromagnetic coupling constant (Georgi, 1981). A change in the strength of the strong interaction coupling constant of only a few percent would have dramatic consequences for the inert and living matters. As small an increase as two percent would block the formation of protons out of quarks and hence the formation of hydrogen atom. Comparable decrease would make certain nuclei essential to life unstable and small changes in the electric charge of the electron would block any kind of chemistry and, by extension of biochemistry (Barrow and Silk, 1980).

2) *Electric dipoles*

In the struggling world of matter-antimatter, the electric particulate partners of the primordial, but potential bipolar couple born from energetic photons, the electron (−) and the positron (+) annihilate one another when they collide, releasing a burst of energy in the form of gamma rays.

Similarly, a proton (H^+) of an hydrogen atom (H) can decay into a positron and a neutral pi meson; the positron would subsequently encounter an electron, perhaps the electron of H, and they would annihilate each other, giving rise to γ-rays, or high-energy photons (Georgi, 1981). This decay of an hydrogen atom into pure radiation represents a conversion of matter into energy. Moreover, when an hydrogen nucleus (proton) is violently collided with a γ-ray, an electron and its antimatter partner, the positron, are created. Because they have the same mass but opposite electric charge, the particulate partners could be visualized as traces curving in opposite directions in the magnetic field of a cloud-chamber photograph (Goldman *et al.*, 1988). Thus, particles and antiparticles can be created as diverging pairs if there is enough energy available to conserve momentum and provide mass ($E = mc^2$) while when particles and antiparticles are free, they can annihilate each other in a burst of energy (Fig. 1A).

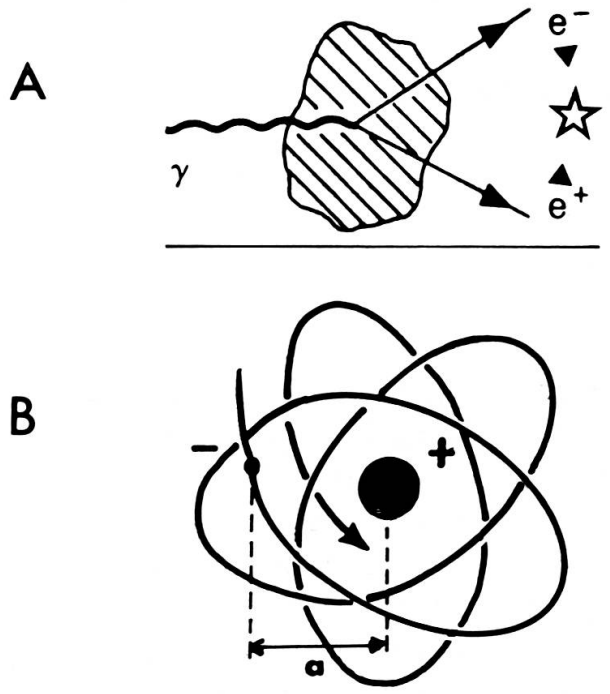


FIG. 1.

(A) Pair production of an electron ($^-$) and a positron ($^+$) by conversion of an energetic photon (γ) in the presence of an external electromagnetic field (dashed crystal); further annihilation of e^- and e^+ with energy burst (☆). Adapted from Sörensen and Uggerhoj, 1987.

(B) Multiple lemniscatic orbitals (wave functions) of the single electron ($^-$) around the proton ($^+$) of the atom of hydrogen (H). Axis (a) of the transient electric dipole probabilistically positioned.

It was only in the realm of stable matter that bipolarity could be actualized when a negative electron was matched by a positive proton, both being neutrally united — and not annihilated — into the first and simplest atom, hydrogen. In it, the single electron is randomly positioned around the unique proton according to the spin-polarized model of Einstein-Bose (Farago, 1974). Its opposite electric charges could thus remain separate by the spinning of the electron on an elliptic orbital maintaining it at distance from the central proton (Fig. 1B). This distance could be visualized as that of the first, probabilistically positioned axis of electric bipolarization.

However, how was it that the proton did not absorb the orbiting electron, leading to the decay of the hydrogen atom into a shower of photons? Puzzled by this question, Weyl suggested, in 1929 already, that the stability of matter might be explained if each kind of charge were conserved separately, the mutual annihilation of a proton and an electron being forbidden. This important question was only taken up again in 1938 by E.C.G. Stueckelberg and by Eugen P. Wigner (in a footnote) in 1949. They proposed what became the conventional views, namely that in addition to energy and electric charge there is another conserved property of matter, which has since come to be called baryon number. The baryons (from the Greek barus, heavy) are a family of particles that includes the protons and many particles heavier than the proton, such as the neutron and the highly unstable particles called hyperons. All baryons are assigned a baryon number of +1, and all lighter particles, including the photon, the electron, the positron, the graviton, the neutrino, the muon and the meson, have a baryon number of zero. According to the law of baryon-number conservation, the total baryon number cannot change. The decay of a proton into a collection of lighter particles would entail the conversion of a state whose baryon number is zero, and so the decay is forbidden (Weinberg, 1981).

3) *Polarized conductivity*

Electrical properties as conductivity and its reciprocal, resistivity, are responses to the stimulus of an electric field. When a voltage is applied across a semiconductor, it subjects the electrons to an electric field and causes them to accelerate. The electrical conductivity in the lattice of a semiconductor crystal of pure silicon oxide (SiO_2) can be precisely controlled by doping impurities: n-type doping is produced by freely moving electrons, for example of an arsenic atom in a silicon crystal; p-type doping of silicon by an element such as gallium, deficient in valence electrons replaced by positively charged holes. Doping of silicon semiconductors confers the desired properties of electrical conductivity across the three adjacent regions of field-effect transistors: the source, the channel and the drain. Thus a small voltage applied to a metal gate above the channel controls the polarized flow of electrons (or of positive holes) from the source to the drain (Mayo, 1986). In the bipolar type of transistor, the electric charge is said to be applied to the base to control the flow of current between the emitter and the collector (Chaudhari, 1986).

The transistors are imprinted on semiconducting chips employed in high-speed logic operations. Chips in a computer's memory store millions of fundamental units of information, i.e. the bits, or binary digits, all equivalent to the result of a choice between two alternatives such as "yes" or "no", "on" or "off" ... and therefore + or −.

Photonic emission can also result from the abrupt change from a p-doped region (valence band) to an n-doped one (conduction band) and thus forms a diode across the p-n junction (band gap). The voltage – stimulated recombination of excited electrons in the conduction band with holes in the valence band then liberates energy as photons of laser light (Rowell, 1986).

The dipole force exerted by light on atoms can be understood by considering them to be polarizable bodies. It is often divided into two parts, the light-pressure or scattering force and the gradient or dipole force (Ashkin, 1980). The optical electric field induces an electric dipole moment in the atom and the induced dipole is acted on by the optical electric field. If the light intensity is spatially nonuniform, a force is induced parallel to the gradient of the intensity. The dipole force attracts an atom to a region of high light intensity if the frequency of the light is below the atomic resonance and repels it if it is above. Sodium atoms have also been trapped, by means of the dipole force, near the focus of a laser beam tuned below the first resonance transition (Chu *et al.*, 1986).

Electric ceramics superconductivity was discovered in 1986 by Bednorz and Müller in Cu-O perovskite-type minerals. These authors proceeded under the working hypothesis that an increase in the density of charge carriers in the materials — either in the form of electrons or positively charged "holes" — would lead to a corresponding rise in the transition temperatures (with transitions ranging from 30 to 110°K). This was found with a certain form of barium-lanthanum-copper oxide ($Ba_xLa_{2-x}CuO_4$).

Perovskites are ceramics described by the generalized formula ABX_3 corresponding to $CaTiO_3$ (calcium titanate oxides in their archetypal rare mineral perovskite). Their unit crystal cell is a cluster of polyhedrons. In the ideal form, the B cations may remain at the center of their octahedrons while in some other perovskites they are slightly shifted. The positively charged cations can thus give crystals electrical polarity: one end is positively charged and the other end is negatively charged. Moreover, the direction of the off-centering can often be changed simply by subjecting the sample to an electric field. Such perovskites thus behave as ferroelectrics that are both polarized and able to reverse polarity under the influence of an electric field. A synthetic ferroelectric perovskite $BaTiO_3$ (barium titane oxide) is widely used in electronic devices such as capacitors (smoothing out uneven flow of current) because of the "off-centering" of its cations. "The stronger the applied field, the more the cations are energized and dispersed and the more strongly polarized

the crystal becomes. When the electric field is removed, the cations return slowly to their normal positions and release the stored energy''(Hazen, 1988).

If the distribution of charges in a ferroelectric-ceramic crystal is not symmetric about the crystal's center, a shift in polarization can also be produced by deforming the crystal. This is the basis of piezoelectric ceramics. When such ceramics are mechanically deformed, they develop a considerable charge; conversely, when they are subjected to an electric field they deform (Bowen, 1986).

Traditional explanations of superconductivity were based on phonon-mediated interactions with electrons (Bardeen-Cooper-Schrieffer (BCS) theory, 1957). They provided the somewhat modernized formula for the superconducting transition temperature (T_c) which makes obvious plasmons as candidates for the mediation of the electron-electron pairing.

Superconductivity has recently been explained by Anderson (1987) as based on resonating valence bonds and relying on the plasma oscillations of electrons or of holes. An alternative approach is that proposed by Mott (1987) of a bipolaron mechanism, whether single-site or not, and driven by electron-phonon coupling. Bipolarons are polarons binding in pairs which can behave as ''bosons'' and undergo Bose condensation into a macroscopic superconducting coherent quantum state (Emin, 1982). Otherwise, the polaron has been described as an important example of a generalized soliton in condensed matter, a notion which has been widely generalized in the past decade, especially by physicists in condensed-matter and field theory (Bishop *et al.*, 1987). An electron-induced local strain in the lattice of an unique crystal has also been referred as a ''polaron'' (Campbell, 1987).

In the most recently found bismuth oxide (Bi_{1-x}, K_x, BiO_3, Cava *et al.*, 1988) superconductivity would also be driven by a ''bipolaronic'' mechanism; the charge-ordered parent oxide could be regarded as a lattice ordering of bipolarons, or two-electron pairs, on alternate Bi^{5+} sites stabilized by oxygen relaxation. These electron pairs would become mobile by potassium-doping effect changing the number of bipolarons per bismuth site. This new system would be the best candidate so far for the long-sought bipolaronic limit of superconductivity (Rice, 1988).

In metallic systems, superconductivity is caused by an attractive interaction mediated by the exchange of lattice vibrational excitations (phonons) between electrons forming coherent pairs in the superconducting state (Bardeen *et al.*, 1957). Single crystals of these copper oxide (cuprate) superconductors, including those of the lanthanum (La)- and yttrium (Y)- barium (Ba)-based materials all show a strong anisotropy in the response of their transport properties to applied magnetic fields. Many have suggested that coupling of the conduction electrons to the magnetic moments in the metallic superconductors could cause the pairing of electrons essential to superconductivity. The superconductivity of metallic systems might thus arise from an essentially magnetically-induced interaction.

The electron-phonon coupling mechanism has quite recently been questioned for oxides of $YBa_2Cu_3O_7$ type. Severe constraints have also been imposed on theoretical models implicating the mode of coupling (Little, 1988). As newly proposed guidelines (Sleight, 1988) to further ascent superconductivity temperature, there are more concern with boundary between localized and delocalized electron behavior and with Cu-O sheet systems because the electrons paired with spins antiparallel (BCS theory) appear to reside mostly on oxygen.

D. Magnetic polarization

Magnets have fascinated man since the observation that iron is attracted to lodestone. Today highly magnetic ferro- (Fe and CrO_2) and ferrimagnetic (Fe_3O_4) materials are used in technological applications. These highly magnetic materials share common structural features such as atomic basis and orbital metal-based spin sites. On these fundaments, even molecular/organic based ferromagnets could be recently developed (Miller *et al.*, 1988).

1) *Cosmological level*

The earth magnetic field is known to be polarized. Recently evidence has been obtained for north-south changes or "reversals" in the polarity of this geomagnetic field. Observations of the intensity and direction of the field during the polarity transition have shown that the intensity decreases by a factor of four and the global morphology of the field becomes largely nondipolar (Jacobs, 1984). It has recently been shown that a patch of flux of opposite sign to that expected for a dipole field occurs beneath southern Africa. Attempts have recently been made (Gubbins, 1987) to answer the question of "How can this rapid fall in dipole moment be related to secular variation of the core field ?"

Magnetic fields could be dynamically important for dense clouds and star formation as shown by Zeeman splitting in OH molecules. They are believed to dynamically decouple from the gap through processes such as ambipolar diffusion of ions through the neutral bulk of the gas. The Zeeman effect must be estimated from the difference in intensity between the right- and left-handed circularly polarized portions of the radiation (Boss, 1987). The magnetic field strength in dense molecular clouds is essential for determining to what extent magnetic fields are dynamically significant during protostellar contraction and collapse, as well as to explain the present chromospheric activity of stars.

2) *Magnetic fields*

The magnetic properties of a solid can be traced largely to the magnetic behaviour of individual electrons or groups of electrons within the solid. Every electron

generates a small magnetic field like that of a bar magnet. When a lone electron is placed in a magnetic field, it is energetically favourable for the electron's magnetic field to be aligned in the same direction as the external field. If a sample of a given material contains a large number of lone electrons that do not interact with one another, then all those electrons will tend to align with any external magnetic field. This effect, which is known as paramagnetism, increases the strength of the total magnetic field within the sample.

There is an obvious analogy between ferroelectricity and ferromagnetism. Ferromagnetic compounds show a strong interaction between the atomic moments and their coupling is so intense that all dipoles are spontaneously oriented in the same direction (spontaneous magnetization of ferrite in the absence of a magnetic field). Diamagnetic compounds lose instantly their magnetization when the external magnetic field disappears while paramagnetic compounds can keep a faint one in the absence of a magnetic field.

Polarized patterns of magnetization have been applied to data-storage technologies for advanced computing. In tapes or disks, the magnetic storage of binary data essentially amounts to impressing a pattern of magnetization on a medium most commonly made of iron oxide. The pattern of magnetization is impressed on the medium as it moves under a gap simply by repeatedly reversing the flow of electric current in the inductive recording head. "Because computer-generated data are stored as a string of binary digits, the pattern of current reversals (and therefore the pattern of magnetization reversals) corresponds to a succession of 0's and 1's that constitute the individual bits of data" (Kryder, 1987). Back consideration from the alternate magnetization of binary digit to electric reversals highlights that the fundaments of advanced computing are deeply rooted in the universal +/- electric bipolarity.

3) *Magnetic monopoles*

In their aim to understand the Universe, the physicists have not only asked the major question "what is matter?" but also "what are the forces that act on it?" In their effort to answer them, they have dwelled on the interaction of particles and fields, particles producing fields which act on particles in a perpetual duet. These notions of "particles" and "fields" that is matter and force, were already at the core of Newton's theory of gravitation and Maxwell's theory of electro-magnetism. Quantum field theory then provided a theory of elemental particles consistent with Planck's quantum mechanism and based on a sophisticated unification of the notion of particle and field. Electromagnetism remains the simplest gauge theory, the generic name for a class of vector field theories. Generalizations of electromagnetism are called non-Abelian gauge theories. It is the topological solution (with non trivial boundary) of certain of these theories which has led, in 1974, to the discovery of generalized magnetic monopoles by 't Hooft and Polyakov (Hey *et al.*, 1988).

The dipolar nature of ordinary magnetic materials can readily be demonstrated with iron fillings sprinkled on a piece of paper held over a bar magnet. Cutting the magnet in half does not isolate the poles; instead two smaller bar magnets are created rather than isolated "north" and "south" poles. By comparison, when an electric dipole is made by depositing electric charges of opposite signs on the ends of an insulating rode which is then cut, two isolated electric poles are created. Such a possibility of isolating electric poles but not magnetic ones is a fundamental distinction between electricity and magnetism (Carrigan and Trower, 1983).

An example of a magnetic monopole would be an isolated pole of a bar magnet. Classically, of course, such objects are not found. Monopoles are even virtually eliminated because over a region only one trillionth the size of a proton, the Higgs field is essentially constant (Guth, 1983). Thus there is the philosophically appealing possibility that the Higgs transition is the origin of all matter and energy. The "universe", notes Guth, "could be the ultimate free lunch".

According to Okun *et al.* (1984), if the theory of grand unification [SU(5)] of the four forces in the universe is correct and the Higgs field (scalar bosons) does exist, magnetic monopoles should have been created in the first 10^{-35} second of the universe. Proponents of the Su(5) theory differ over the internal composition of the monopole and over how many monopoles should exist; it is generally agreed that the monopole should have an enormous mass for an elementary particle, perhaps from 10^{16} to 10^{17} times the mass of the proton.

Although there have since been scattered reports of finding monopoles, none have yet been substantiated. As remarked by Hey *et al.* (1988) "no monopole has actually been observed neither their existence required in the standard quantum chromodynamics electroweak theory. However, they are predicted to exist in more ambitious "grand unified" theories that incorporate the color and electroweak gauge groups into a larger gauge group although the density of such monopoles in the universe may be so low as to make their detection quite unlikely."

4) *Spin polarizations*

Since 1925, it is known that every electron has a property called spin which became an important element in the development of quantum mechanics and atomic physics, particularly in the work of P.A.M. Dirac. A spin is the quantum number of electron. It makes this particle behave like a bar magnet, giving it a magnetic moment (Fig. 2A). Unlike the spin of a ball the spin of an atom or a subatomic particle is therefore quantized.

The magnitude of the spin can assume only integer or half-integer values and the vector can have only a finite number of directions. The hydrogen atom has integer value (1) of spin and belongs therefore, as the photon (0) to the boson class of particles. However hydrogen is a composite boson and its contituents proton and

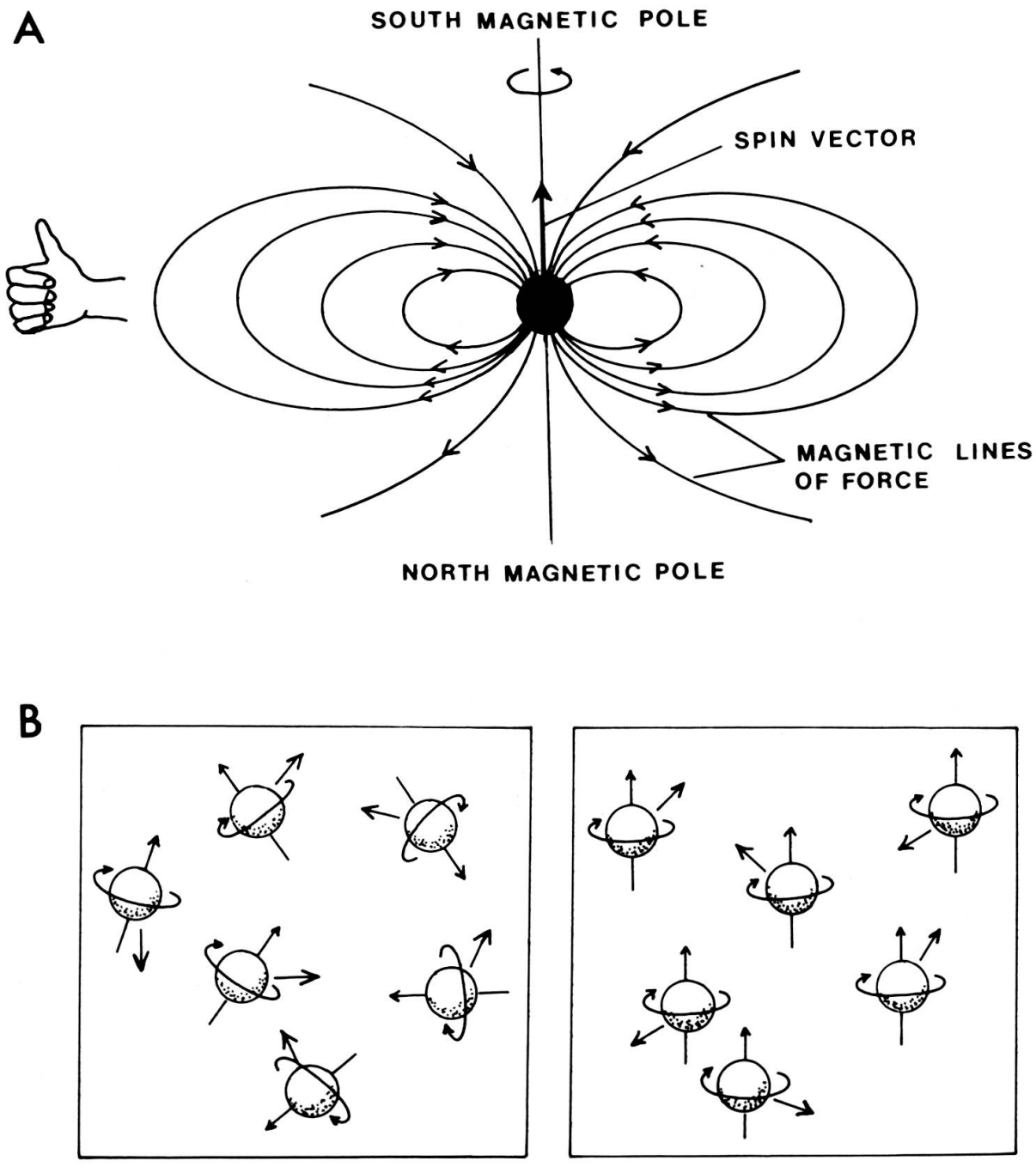


FIG. 2.

(A) Spin angular momentum of a particle with both mass and spin generating a magnetic field. For a negatively charged particle such as the electron the magnetic north pole points in the direction opposite to that of the spin vector (right thumb pointed in that direction). Redrawn from Fig. in I. F. Silvera and J. Walraven, Scientific American, vol. 246 (1), p. 58 (1982), with authorization.

(B) *Left:* gas of unpolarized atoms, with their spins pointing in all directions. *Right:* spin-polarized atomic nuclei, with their spins, or spin angular momenta, aligned in the same direction (arrows pointing up). Redrawn from Fig. in F. Laloë and J. H. Freed, Scientific American, vol. 258 (4), p. 70 (1988), with authorization.

electron which have half-integer value of spin (1/2) are fermions (Silvera and Walraven, 1982). Contrarily to bosons, fermions obey the quantum mechanical rule called the Pauli exclusion principle. According to this principle, two fermions (electrons, protons, neutrons) cannot occupy the same orbit if they have identical quantum numbers.

The scattering of spin-polarized electrons by spin-polarized "one electron" atoms (such as hydrogen or the alkali atoms) can be described as follows: the spin polarization of the electrons and atoms is denoted by specifying the direction of the spin orientation as parallel or antiparallel to an arbitrary axis of quantization. The spin vector can thus point in one of two directions, either "up" or "down". For instance, the two electrons of a helium atom have antiparallel spins and so the magnetic moments cancel. In paronic helium, however, the spins are parallel and the magnetic moments add up (Fig. 2B). Thus each atomic orbit — in succession at progressively higher levels — can accomodate up to two electrons as long as their spins point in opposite directions. However, the Pauli principle could be violated in rare "paronic" states in which two identical fermions would occupy a state simultaneously; one of the orbits would be filled by two electrons whose spins are parallel (Greenberg and Mohapatra, 1987).

All the building blocks of matter — electrons, neutrons, protons and nuclei — seem to be spinning like tops. The spinning is a basic quantum-mechanical property: each particle has a definite amount of spin, or spin angular momentum, just as it has a definite mass and a definite electric charge. "When two spinning particles collide, the direction of their spin can affect how they scatter, just as the "english" on billiard balls can alter their rebound after collision" (Krisch, 1987).

Spin is an intrinsic property of every particle. In general, it is composite since every atom is a composite entity consisting of a nucleus and one or more electrons. Thus, and according to quantum theory, the nucleus has spin, or spin angular momentum, namely it rotates like a top about its axis. As the electron and the nucleus of the hydrogen atoms are both polarized, these atoms can be considered "doubly polarized" (Laloë and Freed, 1988). Their nuclear spins can be either parallel or antiparallel with respect to the spins of the polarized electrons. Molecular hydrogen can therefore only gradually form when atoms with antiparallel nuclear spins become slightly depolarized and thus can recombine with one another (Silvera and Walraven, 1982).

The properties (viscosity, heat conductivity, etc,) of the spin-polarized gas of atoms produced are markedly changed. This leaves open the puzzling question of "how can nuclear spins, which are so weakly coupled to the outside world, so dramatically change the macroscopic properties of a gas ?" (Laloë and Freed, 1988). Spin waves is another spin phenomenon occurring in a polarized gas. It is "a collective oscillatory mode of the nuclear spins, so that the spins process, or rotate, about the

average direction of polarization at a frequency that depends on the amount of polarization" (Laloë and Freed, 1988).

Polarized beams of particles (protons, deuterons and even tritons) are produced by existing polarized ion sources and used as targets in nuclear physics experiments (Krisch, 1987). They are useful to study either the spin-orbit or the spin-spin interaction. In describing the elastic scattering of these polarized particles the observed angular distribution is usually fitted with an optical potential which must contain a spin-orbit term if it is to yield polarizations (Barshall, 1975).

Like all spinning charged bodies, the electron has a certain amount of spin and therefore creates a magnetic field; in some ways the electron can be thought of as a tiny bar magnet. In most stable compounds all the electrons form pairs in which the magnetic fields of the two electrons are aligned in exactly opposite directions. In some molecules, however, there is an unpaired electron. In electron-spin-resonance spectroscopy the investigator gauges how the energy of such unpaired electrons changes in a rapidly varying magnetic field. The results provide clues to the molecular environment of the unpaired electrons (Youvan and Marrs, 1987).

One extremely powerful method to answer questions about molecular structure is nuclear magnetic resonance (NMR). This spectroscopic technique relies on the fact that atomic nuclei with an odd number of nucleons (protons and neutrons) have an intrinsic magnetism that makes each such nucleus a magnetic dipole, in essence a bar magnet. Two fields are applied to cells or any other part of a living organism. The first is a strong magnetic field which causes the nuclear dipoles (H_1, C_{13} and P_{31} nuclei) to orient themselves so that the dipole of each nucleus is aligned either with the field (low energy) or against it (higher energy). The second field applied consists of electromagnetic radiation in the radio-frequency part of the spectrum. With the magnetic field strength held constant and the radio frequency varied leads sometime to the resonance of the nuclei absorbing the radio photons (Shulman, 1983).

By probing the electronic environment of the cations in an electride — ionic salts behaving as anions, the simplest anion being the electron (Dye and DeBacker, 1987) — nuclear magnetic resonance can also reveal how much time the trapped electrons spend near a cation. Lone trapped electrons are paramagnetic — their magnetic fields become aligned with any external field — and so the magnetic field of such an electron near a nucleus will add to external field to produce a greater total magnetic field in the vicinity of the nucleus.

E. Light polarization

The photon is a quantum of light or electromagnetic radiation. It is a massless particle that has no electric charge of its own and that moves (by definition) with the speed of light. The wavelength of the photon is inversely proportional to its energy, so that the photons associated with radiation of short wavelength such as

blue light are more energetic than "red" photons. The highly energetic "blue" photon is therefore the most efficient to remove an outer electron from an atom (resonance ionization spectroscopy).

In quantum electrodynamics, the photon is therefore the intermediary particle which is exchanged at the interaction of two charged particles, such as two electrons. The explanation of this peculiar properties of the virtual photon lies in the uncertainty principle introduced into quantum mechanics by Werner Heisenberg. The uncertainty principle does not invalidate the conservation laws of energy and of momentum, but does allow a violation of the laws to go unnoticed if it is rectified quickly enough.

Energetic photons may convert into electron-positron pairs, but only in the presence of an external electromagnetic field — a process called pair production (Fig. 1A). The basic mechanism responsible for that conversion of a photon into a particle-antiparticle pair is identical to that enabling a charged particle moving in an external field to emit electromagnetic radiation (Sörensen and Uggerhoj, 1987).

Polarization of the photon — the direction of its associated electric field — is, with its intrinsic spin, an important parameter characterizing quantum waves. Phase shifts in these quantum waves have recently been reported to be caused by varying the polarization state of the photons in split laser beams (Rowell, 1986). In Maxwell's theory of electromagnetism photons transmit forces between other particles. The quantum state of a pair of photons is the state in which both photons are linearly polarized along a vertical axis. Another possible state is the one in which they are both linearly polarized along a horizontal axis.

A photon can exist in ambiguous state until measurement is made. If a particle-like property is measured, the photon behaves like a particle and if a wave-like property is measured, the photon behaves like a wave. Whether the photon is wave- or particle-like is indefinite until the experimental arrangement is specified. The quantum state of the photon is fixed if three quantities are known: the photon's direction, its frequency and its linear polarization (the direction of the electric field associated with the photon).

Because the wavelength of γ-rays is very short (about 10^{14} m at 100 MeV), classical wave technique cannot be used to measure their polarization. The kinematics of the particles produced by the interactions of γ-ray photons (Compton scattering and "pair production") can be used, however. The emerging particles (electrons or electron-positron pairs) have azimuthal distribution related to the plane of the electric vector (polarization) of the incident γ-ray (Dean, 1987).

Natural light with its electromagnetic wave lengths in the visible spectrum can also be polarized. Directed through Nicol prisms of Islandic spath (calcite crystals) or thin "polaroid" filters, light is no longer propagated along all axes covering 360° of a plane perpendicular to light direction but only along a privileged plane called polarization plane. Light can be polarized in three ways: when the oscillating electric fields of the beam are directed left and right in the plane of the page the light is

said to be horizontally polarized; if the electric fields are directed up and down in the plane of the page, the beam is vertically polarized; if the field direction moves like a circle, the light is said to be circularly polarized. Either calcite crystals or piles of glass plates can serve as the polarization analyzers, since each of them is much more efficient than an actual polarizing film in blocking photons polarized perpendicularly to the transmission axis (Shimony, 1988).

Many molecules important in plants and other living things will, in solution, rotate the plane of polarization of a beam of polarized light. These optically active molecules or enantiomorphs (see II.D) typically contain at least one asymmetric carbon atom (an atom with four different groups attached to it). In many arthropods, polarization vision is thought to be based on the rhabdomere structure of their compound eyes. The regular closely packed microvilli of rhabdomeres run in a single direction only and perpendicular to the lozenge long axis, suggesting that they operate as polarization analyzers (Marshall, 1988).

II. MOLECULAR DIPOLES AND CHIRALS

A. Electric dipole moments

According to Debye (1912) if the two gravity centers of opposite charges in a molecule coincide in the absence of an external electric field, it is electrically symmetrical; its electric moment, namely the product of the charge by the distance of the charges to gravity centers is null. Oppositely, in the case of a permanent existence of a "dipole", the molecule is asymmetrical. There is a shift between the gravity centers of the negative charges (electrons) and the positive charges (nuclei); such centers constitute, in the molecule, the two poles of opposite charges which together are called the "electric dipole". The molecule itself and the bonding uniting its two atoms are called "polar".

In the lack of a field, the thermic agitation (in gazes and liquids) confers to the vectors (electric moments of the dipoles) all possible directions in the space.

In a field, two phenomena occur:

1) charges are displaced in the molecule and this becomes polarized (if it was not already);

2) dipoles, formed or modified, tend to orient themselves parallely to the direction of the field when thermic agitation allows it.

In the case of a very quick oscillation of the field, as in a light ray, this polarization of orientation cannot occur because the molecular movement is too slow compared to the frequency of the radiation.

In electricity, the dipole generates a *dipolar electric moment* or molecular movement represented by a vector with important orientation and largeness. The dipolar electric moment is the vector $M = Ql$ in which two equivalent punctual Q charges and of contrary sense are separated by a fixed distance l. In an electrical field E, the dipole is submitted to the couple $C = M \times E$ which tends to orient it in the direction of the field. It is the case of molecules of which negative and positive charges have a gravity center which does not coincide. More complicated charge distributions correspond to multipolar moment.

Polar molecules are able to orient themselves under the many external actions. This phenomenon is known as *polarization,* a term which expresses the idea of the disequilibrium of a phenomenon (electric, magnetic, chemical, vibratory) which is not equally distributed in the space but oriented, eventually around poles. For instance, in dielectricals, the term of polarization expresses the modifications of an insulator submitted to the effects of an electric field.

In *spontaneous* polarization, as in the case of ferro-electrical crystals, all dipolar moments are oriented in the same direction. The dipolar electric moment in their molecules results from dissymmetry of their positive and negative charge distribu-

tions. In other materials whose molecules are permanently polarized by chemical forces, polarization can be caused by molecules induced to rotate into the same alignment under the influence of the electric field (see water molecules, II.B1).

In geophysics, the study of disposition of electric charges at the interface of sand and electrolyte has provided *induced* polarization models (Loeb, 1970). Electric current paths were shown to be altered due to polarization of an ore grain. At the beginning of the charging process, the current passes through the electrolyte and the sphere if it is conductive. Then a polarization builds up and, at the end, the electrolyte shell acts as the dielectric of a condenser; the charging current cannot cross it any more, the grain is by-passed, and the particle of polarizable material acts as an elementary electric dipole. By contrast, in absence of polarizing field, the spherical core is surrounded by an electric field which has a spherical symmetry, and the particle does not act as an elementary electric dipole (Bertin and Loeb, 1976).

B. Mineral dipoles

1) *Dipolar water*

Water has many unusual properties (Franks and Mathias, 1982), among which polarity and self-association which have biological consequences in macromolecule hydration and in cell membranes where the polarity of the molecular components is a major factor in determining how they are arranged. No doubt then that "the chemistry of life is water chemistry" as pointed out by Sheeler and Bianchi (1987).

In the cell, water exists in two forms: free and bound. Free water represents 95 per cent of the total cellular water and is the principal part used as a solvent for solutes and as a dispersion medium of the colloid system of protoplasm. Bound water, which represents only 4 to 5 per cent of the total cellular water, is loosely held to the proteins by hydrogen bonds and other forces. It includes the so-called unmobilized water contained within the fibrous structure of macromolecules. Because of its polarity (see below), water can bind electrostatically to both positively and negatively charged groups in the protein. Thus, each amino group in a protein molecule is capable of binding 2.6 molecules of water (De Robertis *et al.*, 1979).

Macromolecules in aqueous solutions may be "vicinally (interface) hydrated" while small solutes (small ions; small non-electrolyte molecules) do not have such type of hydration but have ion-hydration or "hydrophobic hydration". Thus if one accepts that macromolecules are vicinally hydrated while small solutes are not, the following question must be answered "is there a critical size of molecules above which vicinal hydration occurs?" (Drost-Hansen, 1982).

The hydrogen bonds between water molecules "loose" in water freezing to ice (below 4°C) which then increases its volume while decreasing in density (floating icebergs). "Solid water" or ice may occur with a large number of different structures,

such as the high-pressure ice polymorphs and the clathrate hydrates forming a lattice network. Despite its rigid structure, ice has about the same electrical conductivity as liquid water. This would be due to the possibility of proton jumping or tunnelling effect (see p. 38) along immobilized water molecules in the crystal lattice of ice (Lehninger, 1975).

Considering the many different crystalline ices known, and the nature of their structures, Rice (1975) has suggested that a modest arrangement of the positions of a group of water molecules can (and does) generate new minima in the potential energy surface, and that these new minima correspond to qualitatively different connectivities of the hydrogen-bond network. Only such an infinite hydrogen-bond network (Stanley *et al.*, 1981) could be invoked to explain the "memory" of water when tested as a template for antibody molecules (Davenas *et al.*, 1988).

In a water molecule the hydrogen atoms approximate naked protons on the surface of the oxygen atom. The net charge for the molecules as a whole is neutral (same number of electrons and protons). From spectroscopic and X-ray analyses the precise H-O-H bond angle is 104° 45' (Pauling, 1960). It is not absolutely stable but represents an average sharing of electrons and distribution of charges (Salisbury and Ross, 1985). The protons, thus distributed apart on the surface of the oxygen atom, provide a slight positive charge on one side of the molecule. This is balanced by an equal negative charge on the other side of the molecule. Such a polar molecule in which the electrons are asymmetrically distributed has a dipole moment. Thus, although the water molecule is electrically neutral, its partial positive and partial negative charges are separated, with the result that the molecule is an *electric dipole* (Fig. 3A).

Dipolar molecules of H_2O are attracted to each other to give associated clusters of definite structure (Fig. 3A), but of a size which remains undefined as long as the "surfaces" of the associated entities display a polar distribution of positive and negative charges. However, the oxygen atoms which carry the negative charge are very much larger than the hydrogen atoms. One hydrogen atom is situated between two oxygens, and is bonded on one side covalently to one O atom; in a covalent bond, valency electron pairs are shared between two atoms; since the valency electrons of two atoms are involved, the electron doublet is called a shared pair. On the opposite side it is attracted electrostatically by the negative charge of the other O atom and such electrostatic linkage is known as a hydrogen bond (Pimentel and McClellan, 1960). The fact that the hydrogen bond is considerably weaker than the covalent bond is conventionally denoted by O-H--O. As a result of the asymmetric arrangement of the two covalent bonds, the distribution of electric charges within the neutral molecule of water is such that the hydrogen nuclei appear to be positive with respect to the oxygen which has taken up two electrons. Such dipolar structure of H_2O leaves weakly negative regions near the central oxygen atom at the other two corners of an imaginary tetrahedron. Consequently, when molecules of water

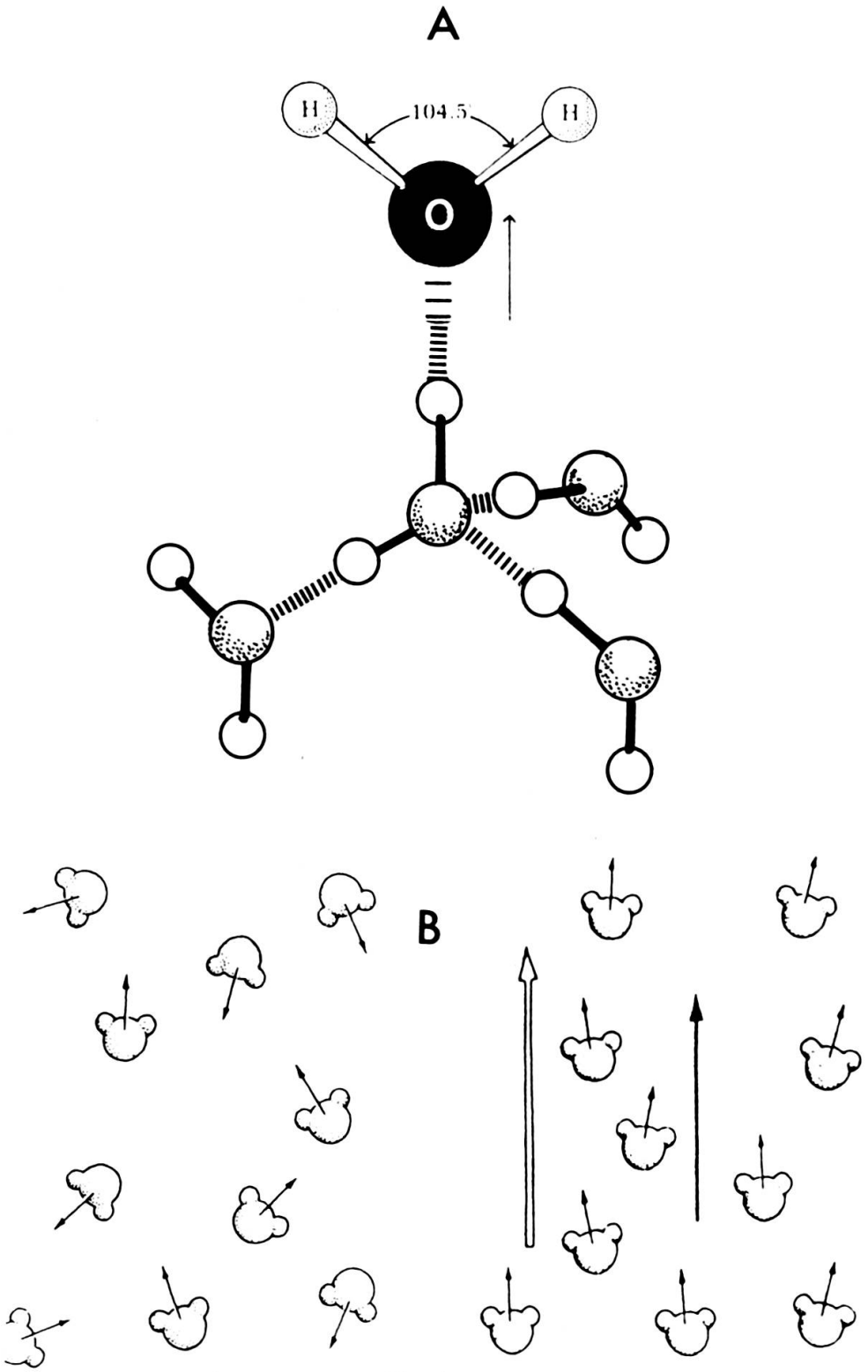


FIG. 3.

Electric dipolar nature of the H_2O molecule.

(A) One water molecule transiently joined to four others in a hydrogen-bonded lattice ("flickering cluster"). Redrawn from "water structure" in Alberts *et al.*, 1983.

(B) Multidirectionally-oriented torques of H_2O dipoles *(left)*. Torques of dipolar molecules all oriented in an electric field *(right)*. Adapted from Walker, 1987.

join together transiently in a hydrogen-bond lattice, one molecule becomes the center of four-components clusters. These small clusters can then join to other water molecules to constitute short-lived assemblies known as "flickering clusters" (Alberts *et al.*, 1983). Liquid water can thus be considered as "a fluid network of transiently hydrogen-bonded molecules eventhough its precise structure is still not known with certainty" (Darnell *et al.*, 1986).

The polarity of water and its hydrogen-bonding property make it a potent polar solvent for many ionic compounds and other polar molecules. Water also disperses amphipathic molecules, such as soaps, to form micelles with hydrophobic groups inside and charged, hydrophilic groups on the external surface. Crystalline salts, e.g. NaCl, readily dissolve in this polar liquid. The Na^+ and Cl^- ions are rapidly hydrated by the dipolar molecules and pulled away from the crystal lattice (Lehninger, 1982).

Among both polar (hydrophilic) and non polar (hydrophobic) types of molecules there can occur a so-called Van der Waals interaction which is considered as a non specific weak attractive force created when two atoms or two molecules such as O_2 approach one another closely. Such weak interaction would result from the formation of transient dipoles because, in any atom, momentary random interaction between transiently forming dipoles results in a net weak attraction (Darnell *et al.*, 1986).

The polarization of the water is expressed as the net dipole moment per unit of volume. It is zero when the dipole moments are randomly oriented, because for every moment pointing in one direction another moment points in the opposite direction. When an electric field begins to align the dipole moments, polarization increases. It would be at the maximum if all the dipoles were in alignment. Random molecular motion or Brownian motion, however, continuously knocks dipoles out of alignment, keeping the polarization below the maximum level. Early in this century the eminent Dutch physicist Peter J. W. Debye demonstrated mathematically why microwave energy is strongly absorbed by water. The key fact is that water molecules cannot rotate instantaneously into alignment with electric field. Since they have a mass spread over a certain volume, it takes time for torque to make them rotate (Fig. 3B). The retarding forces from surrounding molecules also affect rotation. This is the theoretical basis for one of the hypotheses proposed to explain "the secret of microwave oven's rapid cooking action" as being that water in the food rapidly absorbs the energy of the waves (Walker, 1987).

Water has only a very sligth tendency to ionize but the products H^+ and OH^- (10^{-7} M of each at 25°C and pH 7.0) have very profound biological effects. The extent of this reversible dissociation is quantitatively expressed by an equilibrium constant (K'_{eq}, see Lehninger, 1982). However, protons do not exist "bare" in water to any significant extent; they occur only in hydrated form of the so-called hydronium cation H_3O^+ [$= H^+(H_2O)$], which is itself further hydrated through

additional hydrogen bonding with water to form the $H_9O_4^+$ ion, as well as more highly hydrated forms (Fig. 2-6 in Lehninger, 1975).

A proton can jump very rapidly from an hydronium ion to a neighboring H_2O molecule to which it is hydrogen-bonded. Such *tunnelling* has the effect of translocating protons at a rate that is much higher than the rate of diffusive or bulk movement of H_3O^+ ions per se (Lehninger, 1975).

Proton tunnelling may be an important phenomenon in polarized biological systems. It has been proposed that H^+ could jump along the lattice of H_2O molecules hydrating the filaments of actin (Tirosh *et al.* 1980). Hydrated filaments of this contractile protein could thus function as electric "cables" in the polar conduction of protons implied in the internal electric circuitry of apically growing cells (Turian *et al.*, 1985; see VI.A2b).

2) *Other mineral dipoles*

Following H_2O as maximal dipole with a permanent electric moment or Debye unit μ of 1.8, there are SO_2 ($\mu = 1.7$), NH_3 ($\mu = 1.5$), HCl ($\mu = 1.03$) and SH_2 ($\mu = 1.0$). Oppositely, CCl_4 and CO_2 have no electric moment ($\mu = 0$) (Eggert and Hock, 1947). Polar molecules can be associated either in series or as parallel dipoles and quadrupoles. Among the six dimeric species that may be formed from the first row hydrides, HF, H_2O and NH_3 monomers, all the predicted structures illustrated by the Van der Waals stereochemistry were in essential agreement with experimental data obtained by high-resolution spectroscopy, except for the NH_3 dimer.

New results lead to revise the traditional view that the condensed-phase interaction of NH_3 (NH_3 dimers, etc.) are dominated by hydrogen bonding and that NH_3 can be viewed as an amphoteric substance that can donate a proton through bonding (Nelson *et al.*, 1987). Nevertheless, and in agreement with the traditional view, NH_3 has been confirmed as a nearly universal proton acceptor. As dipole it can combine with H^+ to form the cation $(HNH_3)^+$ or NH_4^+. Electrostatically, the cations H_3O^+ and NH_4^+ are thus comparable as the nucleus H^+ is fixed in the same way on both H_2O and NH_3 molecules acting as dipoles.

C. Organic dipoles

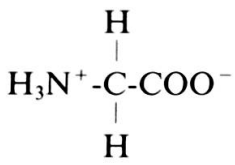
In the more complex polar molecules, dissymmetry of the electronic "cloud" occurs in the same way as in diatomic molecules. The several attractive moments of the atoms for the electrons combine themselves and create a single dipole globally attributed to the whole molecule.

Methane (CH_4) as the primordial molecule of the so-called organic, carbon chemistry is structurally *isometric* and *apolar*: its single C atom shares four electrons

with four H atoms and the shared electron pairs form four covalent single bonds isometrically spaced in tetrahedral arrangement.

Oxidative steps can lead, through the hydrophilic methanol (CH_3OH), to the first negative "monopole" formic acid ($HCOO^-$) while the reductively produced methylamine ($CH_3NH_3^+$) features a primitive positive "monopole".

It is the biologically very significant ability of C atoms to share electron pairs with each other to form very stable carbon-carbon single bonds which has led to *anisometric* molecules, with ethane (CH_3CH_3) as prototype of the hydrocarbon series of homologues. This neutral and hydrophobic molecule has evolved to the primordial biogenic molecules through the hydrophilic ethanol (CH_3CH_2OH) and the electrically *mono*polar molecules (acetic-glycolic-glyoxylic acids) either to the most oxidized, *homo*bipolar oxalic acid ($^-OOC\text{-}COO^-$) or to the primordial amino acid, the electrically *hetero*bipolar glycine as prototypic organic dipole:



1) Single molecules

a) *Small molecules*

Molecules such as CH_4 (methane) and CCl_4 (carbon tetrachloride) are electrically symmetrical and their permanent electric moment or Debye unit is therefore $\mu = 0$. It is in CH_3Cl (chloroform) in which the gravity centers of positive and negative charges are the most distant that the μ ($= 1.89$) is the highest among chloride derivatives of methane (Eggert and Hock, 1947, Tabl. 35). Nitromethane ($\mu = 3.8$), acetonitrile ($\mu = 3.5$) and acetone ($\mu = 2.83$) have the highest electric moments among organic compounds.

Non polar molecules are those with no effective dipole moments. Their bonds are covalent, electrons binding atoms being equally shared as for C-C bonds. The molecules of non polar liquids, like benzene or hexane, show relatively little tendency to attract each other electrostatically. In CH_4 (methane), for example, the carbon and hydrogen atoms have a similar affinity for their shared electron pairs, and so neither the carbon nor the hydrogen atom is noticeably charged.

b) *Fatty acids*

Fatty acid molecules contain both polar or hydrophilic and non polar or hydrophobic parts. In their dissociated states the carboxyl ends of the molecules are mildly soluble in water, whereas the long hydrocarbon chains repel water (Sheeler and Bianchi, 1987).

Like free fatty acids, their salts or soaps, and glycerophosphatides which also contain both polar and nonpolar groups do not dissolve in water, but they do form micelles. A micellar arrangement is not a true solution but a suspension or dispersion. Soap molecules consist of a long, nonpolar hydrocarbon chain terminating in a polar carboxyl group that is ionically bonded to a metal ion such as K^+ or Na^+. When dispersed in water, the soap molecules aggregate to form spherical micelles, in which the polar carboxyl groups of the soap molecules are arranged at the surface of the sphere, where they form weak bonds with the surrounding water, and the nonpolar hydrocarbon chains project inward.

The more complex molecules of glycerophosphatides are characterized as amphipathic, that is, one end of the molecule is strongly hydrophobic (i.e., the end containing the hydrocarbon chains) while the other end is hydrophilic due to the charged nature of the dissociated phosphate group and other substituents (Sheeler and Bianchi, 1987).

c) *Amino acids and amines*

With its simple H- as residue (R) group, glycine is the only α-amino acid having no asymmetrical carbon atom. In the classification of the 20 amino acids according to the polarity of their R groups, glycine belongs to the "polar but uncharged R group" (see below). The high degree of polarity of its α-amino and α-carboxyl groups is not influenced by either a hydroxyl (serine, etc.), amide (asparagine, etc.) or thiol (cysteine, etc.) groups.

Some of the other amino acids are also polar molecules: although they are electrically neutral overall, they have localized concentrations of positive and negative charges. The polarization results from the presence of oxygen or nitrogen atoms, which have a strong affinity for electrons. A few of the amino acids not only are polar but also carry a net electric charge; in other words, they are ionized under physiological conditions. When a polar or charged side chain projects into the aqueous environment, the strongly polar H_2O molecules assume an orderly arrangement.

An approximate classification of amino acids in five groups is based on the degree to which the amino acid's side chain is polarized at pH 7 (Doolittle, 1985): amino acids can be separated into *non-polar* side chains molecules (alanine, valine, leucine, isoleucine); uncharged, *polar* chains molecules (glycine, serine, threonine, cysteine); basic, *positively* charged side chains molecules (lysine, arginine, histidine); acidic, *negatively* charged side chains molecules (aspartic and glutamic acids); other side chains (generally those made up exclusively of carbon and hydrogen) are non polar.

The dipolar nature of amino acids was first suggested by the fact that crystalline amino acids have melting points that are much higher than those of organic molecules of similar size. The crystal lattice of amino acids is held together by strong electro-

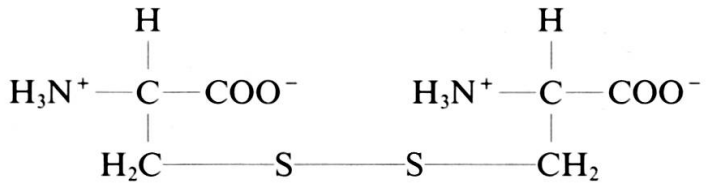
negative forces between positively and negatively charged functional groups of neighboring molecules. When a crystalline polar amino acid such as alanine is dissolved in water, it occurs as a dipolar ion which can act either as an acid (proton donor) or as a base (proton acceptor). Amino acids having this two-way property are *amphoteric* or ampholytes (from "amphoteric electrolytes"). Simple monoamino monocarboxylic α-amino acids such as glycine and alanine are considered as *diprotic* acids when they are fully protonated (see Lehninger, 1982); in this bipolar form they have two groups that can ionize to yield protons (see glycine, p. 39).

Those α-amino acids having a single amino group and a single carboxyl group crystallize from neutral aqueous solutions in a fully ionized species and are called a dipolar ion or zwitterion (German for "hybrid ion"). The characteristic pH at which the amino acid is present as its fully ionized but electrically neutral dipolar form is called the isoelectric pH or isoelectric point (pH_i or P_I). Although such dipolar ions are electrically neutral and do not move in an electric field, they have opposite, equal electric charges at their two "poles".

Bipolarity also occurs in quaternary amines such as the N,N,N,-trimethylglycine or betaine. This molecule is metabolically inert and because of its dipolar nature, no counterions need to be accumulated to maintain electroneutrality when it functions as an important osmoprotective molecule as reported in several groups of Gram negative eubacteria, including halotolerant bacteria, extreme and moderate halophiles, and nonhalophilic bacteria (Hutkins *et al.*, 1987).

2) *Multiple molecules (polar chains)*

Polar molecules can evidently — because of their bipolar fields — associate into multiple molecules that can be described by stoechiometry. There results the formation of polar chains, with a decrease or with an increase of the bipolar state. These associations influence the curve expressing as a function of the concentration, the variations of the orientation polarization of solutions of polar compounds. As prototypic polar chain, the molecule cystine, formed by linkage of two molecules of cysteine via a disulfide bond, is a sort of heterobipolar molecular twin:



By contrast, the molecules involved in primary electrons transfer steps seem to be rather widely separated, by distances of approximately 10 Å. There is no evidence that they are connected by molecular "wires" for electrons to flow through. The active components are rigidly embedded in a non-polar region of the transmembrane protein

matrix. Charge separation, therefore, must occur by electron tunnelling between the components. Quantum-mechanical tunnelling enables electrons to move across relatively large, atomic distances without ever being in between. To elucidate this electron-tunnelling process, compounds are synthesized with barriers to electron transfer deliberately built in — an approach that could be described as molecular engineering. In this spirit, the compound thus synthesized is considered as a device fulfilling a physicochemical function: that of a rectifier for converting electromagnetic waves into a d.c. voltage. The basic design involves a donor group with a low ionization potential, an acceptor with an affinity for electrons, and a hydrocarbon spacer or bridging unit that is (in the first instance) electrically insulating — an inert separator of the donor and acceptor (Fig. 4). The synthesis of such donor-insulator-acceptor devices (DIADs) is now being performed in many laboratories. The most renowned of these compounds are the three-components TRIAD assemblies of Moore *et al.* (1984) composed of carotene, porphyrin and quinone residues.

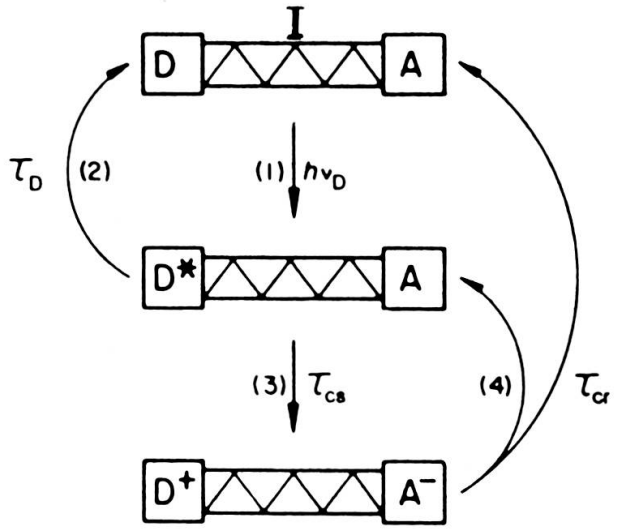


FIG. 4.

Reversible photolytic production of the giant dipole state (D^+-A^-) from a rigid donor (D) – insulator (I) – acceptor (A) molecular device; $h\upsilon_D$ = photoexcitation of the donor moiety; T_{CS} = charge separation; T_{CR} = charge recombination; T_D = lifetime of local-excited-donor to ground-state relaxation. Schemated from Warman *et al.*, *Nature* 320: 615 (1986) and *Nature* 327: 462 (1987).

Joran *et al.* (1987) reported the fluorescence lifetimes of a homologous series of seven porphyrin-quinone molecules, each with differently substituted benzoquinones separated by an identical rigid phenylbicyclo[2.2.2]octane spacer (10 Å, edge-to-edge) which vary with respect to driving force for electron transfer from the first excited single state of the porphyrin. The key features of these series of molecules lie in the fact that the edge-to-edge distance is fixed and exothermicity ($\triangle G$) is tunable.

Thus, the effect of $\triangle G$ on electron transfer can be measured while avoiding major perturbations of the electronic structure.

Many parameters need to be investigated for long-distance electron transfer to be understood and controlled. The role of the driving force for charge separation is clearly of prime importance. Without a driving force, electron transfer could not compete with deactivating processes after excitation by light. However, the energy used in charge separation is lost, unavailable for further chemical or physical work. So it is important to know the minimum driving force necessary to compete efficiently with deactivation. It is significant that for a given compound the rate of charge separation is almost independent of the solvent, be it non-polar benzene or highly polar acetonitrile. This has also been found for the forward electron transfer step of Paddon-Row *et al.* (1986) compounds. The eventual stabilization energy gained from specific ion-polar-solvent interactions in the giant dipole state apparently has little influence on the forward kinetics.

The intensity of charge-separation kinetics to solvent polarity is in sharp contrast with the very strong solvent influence on charge recombination (Wasielewski *et al.*, 1985; Warman, 1987). The rate of long-distance charge recombination increases by two orders of magnitude when the solvent medium is changed from a non-polar saturated hydrocarbon to the more highly solvating ether dioxane (Paddon-Row *et al.*, 1986). This contradicts the intuitive feeling that ionic species are more stable in more polar media, but evidently is highly favourable for photosynthetic compounds working in a non-polar environment. Terms such as "superexchange" and "through-band interactions" have been used in an attempt to describe this non-classical behaviour which may be of particular relevance to the mechanism of photosynthesis. Structural analysis (Deisenhofer *et al.*, 1984) showed that an extra bacterio-chlorophyll and two non-conjugated phytyl side-chains lie along the path followed in the primary electron-transfer steps. This may not be just a coincidence.

Polarization of orientation of the dipole depends upon concentration and provides information about the behaviour of the associated polar molecules. This is examplified by solutions of nitrobenzene in the benzene (non-polar molecules) which present a constantly decreasing polarization of orientation (when concentration increases) while when it is dissolved in ethanol, such decrease is only produced after having passed by a maximum. The study of specific medium effect on the highly exothermic recombination of giant dipoles should be extremely interesting.

D. CHIRAL MOLECULES

The name of chirality is derived from the Greek word for hand and the chiral symmetry has to do with handedness, the property defined in nuclear physics by a particle's spin (see I.D.4) and direction of motion.

Like other symmetries of nature, chiral symmetry has a conservation law associated with it. This law states that the total number of right-handed particles and the total number of left-handed ones can never change. In the ordinary world of protons, electrons and similar particles handedness or chirality clearly is *not* conserved and there is violation of the conservation law. The symmetry-breaking mechanism implies that the weak charge, and the associated handedness of particles, should only be conserved at extremely high energy, where a particle mass is a negligeable fraction of its kinetic energy (Harari, 1983).

"A deeper truly chiral influence comes from the weak interactions, mediated by the recently discovered $W^{\pm}$ and Z^0 bosons. The weak force gives an intrinsic left-handedness, or helicity, to the electron: radioactive β-decay produces an excess of electrons with anti-parallel (left-handed) momentum and spin vectors over the alternative parallel (right-handed) combination, which increases in proportion to v/c, the velocity of light. Similarly positrons emitted in β-decay have an intrinsic right-handedness. Left and right-handed molecules are therefore not true enantiomers, because the weak interaction can distinguish them. The true enantiomer of an L-amino acid is the D-amino acid made of anti-matter. This parity-violating effect of the weak interaction is detectable in a very slight optical activity of atoms" (MacDermott, 1986).

In 1884, Louis Pasteur has conjectured that a dissymmetric force pervades the physical world, extending even to the crystallization dish used for the separation of tartrate enantiomers. He was the first to identify the class of handed or enantiomorphous molecules, which are characterized by structures that are not superposable by translation and rotation alone upon the corresponding mirror-image form. Such structures were first termed "dissymmetric" by Pasteur and, subsequently, "chiral" by Kelvin (1904) from the familiar analogy of the morphological relationship between the left and the right hand. Fischer (1891) then introduced a convention to distinguish between the two mirror-image molecular forms, one being termed the D-isomer *(dextro)* and the other the L-enantiomer *(laevo)*. Although still used for the α-amino acids and the sugars, the Fischer convention accumulated ambiguities of nomenclature and was superceded. In the generally adopted system of Cahn *et al.* (1966), based on the relative mass of the atoms bonded to an asymmetric carbon atom or other chiral centre, the absolute structure of a given chiral molecule is unambiguously specified as that of the R configuration *(rectus)* or the S configuration *(sinister)*.

A stereochemical feat has challenged the general principle originally enunciated by Louis Pasteur. In the so-called *la coupe du roi,* each apple yields a pair of homochiral segments, the two being identical in the ideal case of the sphere (Horeau, 1937). The particular handedness of the two segments depends on the pair of opposite equatorial quadrants chosen for the horizontal cuts. A left-handed segment, given by one of the choices, and a right-handed segment, provided by the other, cannot

be reassembled to form an apple. According to Pasteur (1884), a chemist without dissymmetric agents is able to synthesize only an equimolecular mixture of R and S isomers, a racemic mixture, or an internally compensative single product, with bonded R and S moieties, the so-termed *meso-substances.* Similarly, the chemical degradation of a large achiral molecule would produce, at most, a racemic mixture of chiral fragments (achiral *meso*-2,3-dimethyltartaric acid R- + S- lactic acid). The homochirality of the two halves produced by *la coupe du roi* has as chemical analog the reductive cleavage of an achiral diether which contains *meso*-bridges. "The thought-experiment of Horeau does not violate Pasteur's principle, since a dissymmetric agent is involved, the chiral stereospecific catalyst" (Mason, 1983).

The left-right handedness or chirality is one of the strangest things about the universe. It ranged from the preponderance among spiral galaxies of rotation to the left with respect to the direction of recession to the fundamental question of why life is based on DNA made itself of D-sugar and proteins made of L-amino acids, rather than the enantiomeric (mirror-image) L-sugar/D-amino-acid system. The fact that the L-amino acids and the L-polypeptides are more stable than their D-enantiomers might be a first answer. This could take into account quantifiable disordering effects, such as the known rates of spontaneous racemization of amino-acid enantiomers, and the minor enantiomeric photodiscrimination of solar radiation at twilight, when there is a small net circular polarization, oppositely handed at dawn and dusk (Kondepudi and Nelson, 1985). The slight circular polarization of sunlight around sunrise is balanced by an equal and opposite polarization around sunset, "so this mechanism would only be viable if life evolved in a lake bounded to the east or west by a large mountain" (MacDermott, 1986). Circularly polarized photons would thus cause a similar chiral selection, but without the need for a nearby radioactive source.

The discovery, in 1956, of the non-conservation of mirror-image symmetry in the weak nuclear interaction supported Pasteur's surmise. "The parity-violating weak neutral current interaction gives rise to an energy difference between a chiral molecule and its mirror-image isomer, resulting in a small stabilization of the L-amino acids and the L-peptides in the α-helix and the β-sheet conformation relative to the corresponding enantiomer" (Mason, 1985). The subsequent unification with electromagnetism has allowed the characterization of both the sign and the magnitude of the universal chiral electroweak interaction. The sign is in accord with the observed products of natural selection of enantiomers of one hand from the heterochiral mixture of a racemic substrate, and for the propagation of optically pure homochiral products. In 1953, Frank was surprised to learn, that the origin of biomolecular handedness still appeared to be problematic. He then proposed a homogeneous chemical kinetic mechanism for an open flow-reactor system with a continuous input of achiral substrate from which both enantiomers, L and D, are formed. Each of

the optical isomers autocatalyses its own production and competitively inhibits propagation of its enantiomer.

Life processes on a molecular scale take place between chiral molecules in a chiral environment; presumably, this has been so since primordial biotic times. How did the selection (or production) of one enantiomer of a chiral pair of molecules take place in a prebiotic world where presumably ordinary chemical processes had no statistical preference for selecting (or producing) one isomer over the other? Ordinarily, chemical reactions are indifferent to chirality. However, a reaction such as the formose reaction (formaldehyde polycondensation into monoses) that produces chiral molecules is expected to make racemic mixtures with "left-handed" and "right-handed" forms of the products equally represented. If biochemistry is different in this respect, and if cells can treat enantiomers differently, then this is because the molecules in cells, especially the enzymes, are already chiral. In the evolutionary process, RNA has been suggested as the original genetic material. Since, RNA has been found to function as an enzyme, a new principle which throws light on early evolution (Cech, 1986). The substrates for oligomerization of the first informational RNA molecules could have been prochiral. Thus they gained a chiral centre on oligomerization and molecular-dynamics simulations led to predict a flexible (pyrophosphate) backbone permitting incorporation of monomers in either syn-L-like or anti-D-like conformations without chain termination.

Bernal (1951) suggested that quartz crystals in the primitive environment might have biased a mixture of molecules locally. Quartz crystals come in "left-handed" and "right-handed" versions which are self-seeding; so quite extensive regions might well have contained only one of the kinds of quartz, and hence tended to accumulate or destroy enantiomers preferentially. The units out of which quartz crystals are built — silicic acid — are achiral, so in making a quartz crystal chirality is literally being generated. Presumably in any particular case this is from an initial chance that might have gone either way: "an initial seed happened to start, say left-handed quartz and it simply went on that way" (Cairns-Smith, 1982).

Bonner (1972) has reviewed the long history of this fundamental question of origin. In terms of the doctrine of chemical evolution there are two sorts of explanations that have been given: an external "abiotic" explanation and an internal "biotic" explanation, that is:

1. The environment created an asymmetric bias: in particular, at least somewhere, it provided especially L-amino acids and D-sugars.

2. The environment always produced racemic mixtures of chiral molecules, but at some stage in their evolution organisms came to select molecules of uniform chirality from these mixtures.

Asymmetric synthesis by the use of asymmetric catalysts must also be deliberately considered along with other available methods as a practical strategy for the synthesis of only one of two possible stereoisomers (Mosher and Morrison, 1983). In laboratory

experiments circularly polarized light can show small discriminations in the photochemical decomposition of chiral organic molecules. But it is doubtful if there was much circular polarisation of light in primordial skies: the effect in any case could only have been very feeble.

Other asymmetric influences have been considered. Circulatory polarized photons such as "Bremsstrahlung" γ-rays are generated when longitudinally polarized β-decay electrons interact with matter. Thus, it has been suggested that a fundamental asymmetry of matter — expressed in the non conservation of parity — might have left its mark at the chemical level: "Bremsstrahlung" photons are always polarized the same way. It has been further shown (Bonner *et al.*, 1975) that longitudinally polarized electrons can have a different effect on enantiomers. But again the effect found was small — a 1% enantiomeric bias in the destruction of D,L-leucine.

According to Cairns-Smith (1982), the most radical cause that has been suggested for a possible asymmetry in the early environment is that enantiomers are not, after all, chemically equivalent, only very nearly so (Yamagata, 1966). The idea here is that the fundamental asymmetry between matter and antimatter might show up directly, creating differences in the energies of enantiomers (the true enantiomer of, say, L-alanine being D-alanine made of antimatter). For example, reaction rates, melting points and solubilities might be slightly different.

So where does this lead us: supposing that there was a primordial soup, was it chirally biased? "I don't know" is clearly the best answer, according to Cairns-Smith (1982).

Recently, attention has been called by Rubenstein *et al.* (1983) to synchrotron radiation emanating from supernovae as source of circularly polarized light capable of inducing asymmetric photochemical reactions of prebiotic molecules. This light is predominantly polarized in the plane of motion of fast electrons orbiting around supernovae and when viewed off-axis the polarization is elliptical or circular. Chiral molecules in the interstellar medium or on planetary surfaces would undergo preferential photosynthesis or photolysis when irradiated by such a source. Domains on opposite sides of the plane of predominant polarization would be exposed to light of opposite helicity so that overall symmetry would be preserved. But in a single location, one enantiomer would predominate.

Molecular chirality can be approached either as an expression of random fluctuation or as the result of a specific mechanism. Mann and Primakoff (1983) point out that statistical fluctuation as the basis of chiral dominance is highly unlikely because it would require the assumption of a very small number of terrestrial polymerization sites. The physical processes that have been invoked include the interaction of a racemic mixture with electrons of specific helicity emitted by a nuclear β-decay process, and the apparently more effective interaction of circularly polarized light (CPL) with molecular orbitals. Photosynthetic and photodestructive reactions with

CPL have been described in a number of reviews, and a 2.50% optical enrichment of a racemic mixture of the amino acid leucine has been achieved by photolysis with 212.8-nm CPL. The sources of CPL previously considered include sunlight reflected at the Earth's surface, and by the Earth's magnetic field (Flores *et al.*, 1977). Alignment of interstellar grains has also been identified as a source of linear polarization, and a phase shift can lead to circular polarization (Savage and Mathis, 1979).

It has also been thought that polarized X-rays produced from β-particles could have a role in the origin of homochirality and primeval life processes. Asymmetric decomposition of racemic mixtures of biomolecules by longitudinally polarized β-rays has been described as a possible mechanism. However, the exceptional prebiotic conditions required "do not favour asymmetric β-radiolysis as a selector of the exclusive signature of optical activity in nature" (Meiring, 1987). For life to arise from chiral building-block molecules modified by synchrotron light, it would be necessary for these either to survive intact during the heating process of planet formation or to be ferried on incoming grains as the planet periodically traverses clouds of interstellar material or to be assembled on tepid planetary surfaces illuminated by residual supernova synchrotron radiation.

As already described above, the slight circular polarization of sunlight around sunrise is balanced by an equal and opposite polarization around sunset. Recently, measurements of a net circular optical polarization in sun's light have led Kemp *et al.* (1987) to wonder whether a quite small circular polarization might have influenced the chirality (average handedness) of the Earth's biomolecules: "if biogenesis began not slowly but in a burst of photochemistry, over a period of months perhaps, then a net circular polarity in sunlight might have promoted chirality".

III. MACROMOLECULAR POLARITIES

A. FREE MACROMOLECULES

1. NUCLEIC ACIDS

a) *Deoxyribonucleic acid (DNA)*

a[1] *Structure*

According to the celebrated model of Watson-Crick (1953), both single strands of the antiparallel DNA helix have inverse polarity (5'→3' and 3'→5', Fig. 5).

The two major A and B conformational DNAs are known to depend on the salt content of the fiber while the C conformation is a low salt form of sodium DNA. The recently studied H-DNA is a structure containing both triple-stranded and single-

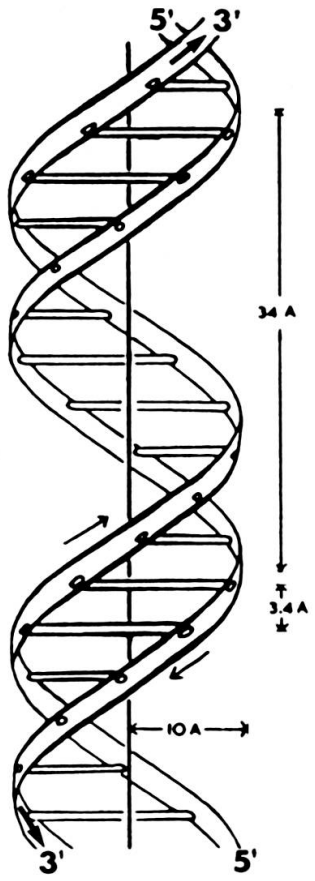


FIG. 5.

Inverse polarity of the two strands (reading direction, 5'-3' arrows) of the double DNA helix. The hydrogen-bonded purines and pyrimidines of the ladder's bars are also poles of a complementarity. After Watson and Crick, 1953.

stranded regions which have been modelled on the basis of reactivity and topological arguments (Htun and Dahlberg, 1988). A prediction of this model is that DNA should introduce a severe kink in DNA molecules which could serve to bring DNA binding proteins into proximity with each other. A conversion from H to B form was found to occur during electrophoresis at progressively higher pHs.

There are three major families of A-, B- and Z-DNA duplexes (Dickerson *et al.*, 1982), which have a common feature, the antiparallel disposition of their constituent strands (Saenger, 1984). Normal Watson-Crick base-pairing is incompatible with parallel stranded duplexes. Recently, however, alternative conformations of DNA molecules have been produced in which hemiprotonation and protonation stabilizing bindings have rended feasible the existence of parallel stranded double helices (Van de Sande *et al.*, 1988). Two of the four hairpin deoxyribonucleotide molecules synthesized "contain either a 3' -P-3' or 5' -P-5' internucleotide linkage in the loop, so that the strands in the stem have the same, that is, parallel polarity". This reversal of polarity necessitated some steps of chemical synthesis in the 5' to 3' direction, that is, opposite to the conventional 3'-5' polarity (Van de Sande *et al.*, 1988).

The classic B-DNA double helix (Watson and Crick, 1953) in its lattice configuration (stable at high relative humidity) consists of two *right*-handed helical polynucleotide chains of opposite polarity — the internucleotide linkage in one strand is 3'→5' and in the other 5'→3'. The pairing rules require that the bases in the two chains are complementary and the nucleoside pairs in the interior of the helix have the planes of their aromatic rings at right angles to the helix axis, and their apolar bulk stacked on top of one another (Drew *et al.*, 1988). The highly negatively charged phosphodiester backbone conversely faces outward, and its strongly polar groups can thus interact with the components of the aqueous environment (Mahler and Cordes, 1969). Oppositely, the helical Z-DNA, discovered by Rich and his colleagues (Wang *et al.*, 1981), characterized by a back-bone which zigzags down the molecule, has a *left*-handed conformation. Chiral metal complexes have provided very sensitive probes for both these left- and right-handed helical structures (Barton, 1986). The DNA double helix is not a regular, featureless barberpole molecule (Privé *et al.*, 1987). Different base sequences "have their own special signature, in the way that they influence groove width, helical twist, bending, and mechanical rigidity or resistance to bending. These special features probably help other molecules such as repressors to read and recognize one base sequence in preference to another".

Polarized unique sequences between inverted repeats have been determined by restriction fragment analysis of chloroplast DNA molecules. A large inverted repeat segment, characteristic of the circular chloroplast genome, has been considered as responsible for a number of its physical properties, among which formation of head-to-head dimers and copy-correction between the inverted repeats. However, "one property which an inverted repeat might be expected to confer — reversal of polarity

of the single copy sequences located between the repeats — has not yet been demonstrated for the chloroplast genome" (Palmer, 1983).

For its central functions of storage and retrieval, DNA can be quite adequately approximated as a one-dimensional line of nucleotides. However, this approximation does not suffice to explain transcriptional regulation. In some regulatory systems, the DNA loops out of one dimension to bring two specific well-separated DNA sites into close proximity (Ptashne, 1986).

Palindromic sequences, long or short, have been considered as being looped out of the linear double-helical structure of DNA. The hairpin(s) or stem- and loop structures could serve as special recognition sites on DNA. In testing ideas about the looping-out process, a fully palindromic circular DNA with head to-head dimer was constructed; linearization (endonuclease) — annealation — ligation then gave a polarity mixture of head-to-tail, head-to-head, and tail-to-tail oligomers (Gellert *et al.*, 1978). DNA gyrase catalyzes a supercoiling reaction which has an intrinsic directionality. In a mechanical model, it left the DNA with a net deficit of helical turns or negative supercoiling (Gellert *et al.*, 1978). In an alternate version of the model, the DNA is translocated without rotation over the enzyme bound to a second DNA segment. The enzyme-DNA complex is rotated by the translocation and "if the polarity of translocation is such that the nicking-closing function sits in the over-wound loop and relaxes those turns selectively, a negatively supercoiled molecule will once again result" (Gellert *et al.*, 1978). Another model was proposed (Liu and Wang, 1978) in which the polarity of translocation is determined by a superhelical wrapping of DNA on the enzyme. However, Gellert *et al.* (1978) preferred to "consider the polarity as arising from the arrangement of the enzymatic sites controlling the rolling motion".

The formation of positively and negatively supercoiled DNA loops by tracking is an example of distant action along a DNA. When both positively and negatively supercoiled loops are generated at the same time by a tracking process, DNA topoisomerase I and DNA gyrase may act differentially in the loop (Liu and Wang, 1987; Wang and Giaever, 1988). Moreover "because of the dyad symmetry of the DNA double helix, the directionality of tracking is usually determined by the asymmetry of the polymerase protein" (Wang and Giaever, 1988).

There is a possibility that solitons (solitary waves) play a role in the properties of DNA and provide a mechanism for transferring energy in that macromolecule (Maddox, 1987). As localized non linear waves propagating and interacting-like particules, the solitons simultaneously create both the environment necessary to support excitation energy or charge, and the conditions for its translation. This proposal is reminiscent of the solvated electron concept in which the electron couples with adjacent water molecules to form a comparatively stable "polaron". The effectively one-dimensional nature of DNA permits a translational wave to propagate, so transferring the energy either as an exciton (coupled electron-hole pair)

or as a charge (polaron). Energy localization, and hence damage, "may result from the collapse of the soliton at some discontinuity in the structure of the DNA molecule, such as a B/Z transition, an intercalated or otherwise bound molecule (particularly with energy or charge accepting character) or as the result of the coincidence of two passing solitons" (Baverstock and Cundall, 1988).

a^2 *Replication*

The macromolecule of DNA can be considered as a duplex of two inversely polarized strands distinctive by their 5' or 3' strands. DNA synthesis is highly asymmetric. This asymmetry is inherent in the opposite-running polarity of the complementary DNA strands: the two complementary DNA strands run in opposite directions (3' to 5' on one strand, 5' to 3' on the other), and the two branches of the DNA replication fork are not identical; one strand, called the lagging strand, makes short fragments of new DNA that subsequently are joined into a continuous strand, whereas the other strand is elongated from the outset as a continuous piece. Replication proceeds polarly along both template strands in the 3'- to -5' direction and, therefore, the process is discontinuous for one of the strands.

The circular bacterial chromosome is replicated semiconservatively and *bidirectionally* starting from a single origin point. DNA polymerase I of *E. coli* makes a strand of DNA complementary to the template strand and the polarity of the new strand (chain growth in the 5'- to -3' direction) is opposite to that of the template strand. The leading strand of DNA is replicated continuously in that 5'- to -3' direction whereas the lagging strand is replicated in short pieces called Okazaki fragments made in the direction opposite to that of the movement of the replication fork (see Lehninger, 1982).

Polarity of DNA replication has recently been shown not to change as a function of transcriptional activity (James and Leffak, 1986). This finding is consistent with earlier suggestions that DNA replication in the transcriptional direction may be a necessary but not sufficient condition for gene expression. Replication proceeds from upstream (5') origins through the alpha-globin locus irrespective of transcriptional activity in erythrocytes (James and Leffak, 1986). Active genes are replicated in the transcriptional direction and cells may switch the replication polarity of genes in concert with gene activation or repression. The replication in the early synthetic (S) phase of genes whose transcription is activated by *trans*-acting factors and/or chromosome translocation suggests that protein binding and chromosome position may also influence replication polarity (Calza *et al.*, 1984).

a^3 *Transcription*

Transcription begins when an enzyme called RNA polymerase binds to a specific base sequence on DNA, the promotor. The RNA molecule always polarly grows in

the 5'- to -3' direction. The polymerase continues along the DNA chain until its passes another special base sequence, the termination signal (Darnell *et al.*, 1986).

In prokaryotes, the degree of supercoiling of DNA can profoundly influence the use of specific promoters. DNA topology and supercoiling can be important parameters when considering DNA wrapping around histones into a left-handed toroidal coil as topologically equivalent to DNA writhe having a negative sense (Drlica and Rouvière-Yaniv, 1987). Supercoiling or topoisomerase activity or both can affect numerous chromosomal processes, such as replication, transcription, and recombination (Bjornsti *et al.*, 1986). Thus, DNA in bacterial cells would be under negative superhelical tension arisen from the action of a topoisomerase (Drlica and Rouvière-Yaniv, 1987). In eukaryotes, a variety of indirect observations suggest that DNA topology has a similar importance in proper gene expression. Much attention has therefore been focused on the cellular proteins that control DNA supercoiling, among which are the enzymes topoisomerase I and II.

The higher order intertwining of DNA generally takes the form of supercoiling of the helix axes. Catenation-induced supercoiling occurs by the wrapping of one DNA duplex around another in process involving chirality: left-handed and right-handed toroidal catenanes were observed (Wasserman *et al.*, 1988). While there is left-hand coiling of DNA around histones in the nucleosomes (Germond *et al.*, 1975), intertwined catenanes of DNA substrates containing bacterial and λ-phage attachment sites are exclusively right-handed (Spengler *et al.*, 1985).

Peptides with an excess of negatively charged side chains forming "acid blobs" can stimulate the formation and/or action of transcriptional preinitiation complexes. There is wide variety of such transcriptional-regulatory proteins that bind to specific DNA targets called "enhancers" or "upstream activating sequences". According to Sigler (1988) "The position and polarity of these *cis* regulatory sequences are not stringently defined with regard to the promotor that they activate".

The traditional view of transcription of bacterial operons is that they are synthesized into long polycistronic mRNAs — which represent the sequences of all the genes of the operon. The messengers are sequentially translated into the various proteins for which they code. Termination of DNA transcription has been considered as a plausible mechanism for controlling gene expression during phage infection. This termination is catalysed by one of the innumerable protein factors of macromolecular metabolism in bacteria, the *rho* factor known to act as a template of phage DNA. The immediate significance of *rho*-dependent termination within an operon is that this mechanism may thus explain the natural polarity of some operons, in which later genes direct synthesis of less protein than earlier genes.

Polarity of transcription-translation is revealed when a mutation which prematuraly interrupts translation of any gene of a typical bacterial operon is apt to depress the translation of all genes which come later in order of transcription. The strength of this polar effect depends, in general, on the distance between the

mutant chain-terminating codon, and the normal terminator at the end of the gene — the less this distance the weaker the polar effect tends to be. Both these phenomena — coordinate regulation and polarity — have often been interpreted as meaning that the operon is a unit not only of transcription but of translation as well.

Polarity in prokaryotic organisms reflects their obligate coupling of transcription to translation. By contrast, in eukaryotes, translation occurs in the cytoplasm on monocistronic units of mRNA and reduction of distal expression would be differently operated (Bigelis *et al.*, 1977; Steege and Söll, 1979).

a[4] *Mutations*

Polar mutants are due to base substitution in the DNA, but instead of coding for a different amino acid, the altered triplet now codes for a chain termination codon, i.e. a triplet preventing further translation in a strand of messenger RNA.

Mutations which prematurely interrupt translation of any gene of a typical bacterial operon are apt to depress the translation of all genes which come later in order of transcription. The strength of this polar effect depends, in general, on the distance between the mutant chain-terminating codon, and the normal terminator at the end of the gene — the less this distance the weaker the polar effect tends to be.

The presence of nonsense mutations and the insertion of foreign DNA into the operon are also a cause of polarity. Not all nonsense mutations are polar and, in general, only those at the 5' ends of a given gene are extreme polars, with a gradient of polarity extending toward the 3' end (Watson, 1970). As for insertion mutants, they differ from nonsense mutants in that they are always extremely polar and their polarity does not show the dependence on position within the gene characteristic of nonsense mutations. The polarity of nonsense mutations might be explained by a similar mechanism to that of polarity resulting from *rho*-dependent termination because nonsense codons constitute part of the DNA sequence recognized by *rho* factor. However, polar nonsense mutants in the galactose operon proved to have no effect on transcription in either the absence or presence of *rho*. The polarity of nonsense mutants would thus depend on the failure of translation as such and not on the sequence of the nonsense codons themselves.

Certain mutations in the structural gene of an operon have a dual (pleiotropic) effect. As explained by Mahler and Cordes (1969) "they not only control the structure of the resultant polypeptide but they also effect a decrease in the amount of all enzymes controlled by genes located beyond the gene primarily affected and distal from the operon (-4-3*-2-1-*0*). Moreover, mutation (*) in 3 will affect the amount of enzyme produced by 4,5, etc. Such polarity mutations were first observed in the *His* operon. Only the genes away from the operator were affected. Polarity mutants have been observed in other systems among which the best studied *Lac* operon (Mahler and Cordes, 1969): mutants in the *z* gene produce less of the enzymes con-

trolled by *y* and *x*, and mutants in *y*, the permease gene, produce decreased levels of transacetylase *(x)* but have no effect on galactosidase *(z)*.

Polarity is also revealed by observation of polar effects of nonsense mutations on distal gene expression. For example, when an operon such as *trp* (tryptophan) is transcribed from its normal promotor, amber mutations in the *tryp E* gene can sharply reduce expression of downstream genes. The reduced yields of the distal polypeptides of an operon could be a direct result of altered translational controls; alternatively, they could simply be a consequence of the fact that distal mRNA templates are not present for translation. Evidence for this last proposal has been obtained with the *E. coli* tryptophan operon in which the limitation in gene expression distal to a polar expression derived from the markedly reduced levels of the relevant mRNA sequences (Morse and Yanofsky, 1969; Steege and Söll, 1979). Inversely, polarity supressors selected to relieve mutational polarity in both the *tryp* and *lac* operons simultaneously increased wild type *tryp* mRNA synthesis (Korn and Yanofsky, 1976; Steege and Söll, 1979).

The recently studied crystal structure of the *trp* repressor/operator complex has shown an extensive contact surface including solvent-mediated hydrogen bonds to the phosphate groups of the DNA; no direct hydrogen-bonded or non-polar contact with the bases were detected. This absence of direct hydrogen-bonds implies that "in *trp* system, specificity is not due to direct hydrogen-bonded contact of the major groove's polar groups by the hydrogen bonding groups of the protein" (von Hippel *et al.*, 1986; Otwinowski *et al.*, 1988). A certain degree of helical twist to accomodate both polar and non-polar stabilizing contacts is needed. Three polar contacts mediated by well ordered water molecules bridge the functional groups of mutationally sensitive base pairs to the protein and thereby contribute to "direct readout" of the operator sequence (Otwinowski *et al.*, 1988).

Transposition mutagenesis by so-called transposons has been used successfully for the genetic analysis of cloned genes. As with transposons, insertional mutagenesis with a selectable DNA fragment or interposon has strong polar effects in a wide range of bacterial species. The strong polarity of the interposon mutation manifested in Gram-negative bacteria was probably due to their flanking *rho*-independent transcriptional terminator sequence which comes from a bacteriophage T40 gene (Prentki and Krisch, 1984).

Forbidden inversions have recently been described in the polar rearrangement of the chromosome of enteric bacteria: "sequences at many pairs of sites sequences (permissive) do recombine to generate the expected inversion, while the same sequences placed at other pairs of sites (non permissive) do not form an inversion" (Segall *et al.*, 1988).

a[5] *Gene conversions*

There are two major mechanisms for the control of gene expression: DNA inversion and DNA transposition. In the first or flip-flop control principles, gene expression is either controlled by an invertible DNA segment or activated by the transposition of a copy of the silent gene to expression site, where it can be transcripted (Borst and Greaves, 1987). The duplicative transposition occurred by a gene conversion, in which a gene in the expression site was displaced by the incoming gene copy and destroyed ("cassette model", see Hicks *et al.,* 1977). Duplicative gene conversion can result in the switching of yeast mating-type; in this event, there is replacement of the α sequence of MATα with *a* sequence from HMR*a*.

In certain eukaryotic genes, called polarons, there appears to be a gradient of conversion frequency from one end of the gene to the other as discovered in the fungus *Ascobolus* (Lissouba and Rizet, 1960, in Fincham *et al.,* 1979). The more frequently observed polaron effect is a strong tendency, in inter-mutant crosses, for recombinants to arise by conversion of the more distal (or in other polarons the more proximal) site rather than the other one. According to Fincham *et al.* (1979), "the polaron effect can be accommodated within a hybrid DNA hypothesis by making the additional assumption that hybrid DNA is not formed in a completely random way but tends to be initiated at certain fixed points, if at different points, within rather well-defined short regions".

Among tetrads in which wild-type segregants have arisen by nonreciprocal interallelic recombination it is often observed that conversion occurs at different rates at the two heterozygous allelic sites. More surprisingly, observed conversion rates frequently depend less upon the specific site undergoing conversion than upon the particular second heterozygous site with which it is associated (Emerson, 1966). The most striking examples of this sort have been found at loci affecting ascospore color in *A. immersus,* in which there exists a strongly polarized control of gene conversion (Rizet's group, 1960, 1961, in Fincham *et al.,* 1979). Complete polarization may exist in a large section of a polaron gene. For a given locus, six allelic sites constituting such a polaron were mapped in linear order by the frequencies of recombination occurring between each pair of sites.

In *Neurospora crassa* there are also indications of polarity in interallelic recombination between members of two groups of sites within a locus (Case and Giles, 1958, 1964; Stadler and Towe, 1963), but to a much lesser degree than in *Ascobolus.*

b) *Ribonucleic acid (RNA)*

RNA is involved in many biological functions, ranging from information storage and transfer to the catalysis of reactions implicating both nucleic acids and proteins.

Transcription normally occurs from DNA in which it begins where an enzyme, RNA polymerase, binds to a specific base sequence (the promoter) on one strand

of the DNA double helix. The RNA molecule always grows in the 5'- to 3' direction. The sequence TATA is involved in positioning the RNA polymerase correctly and transcription of DNA begins between 20-30 nucleotides since past that site. A chemically protective "cap" consisting of a methylated guanosine is linked to the first nucleotide by a triphosphate bridge at the beginning (5'-) of the chain in all eukaryotic messenger RNA (mRNA).

In the problem of recognition between mRNA and amino acyl- *t*RNA, one of the questions arisen is "what is the actual sequence of bases for various codons?". Its answer is that polarity is involved in the highly specific selection of the sequence isomers and their recognition examplified as follows (Mahler and Cordes, 1969): "G_pU_pU induces Val-*t*RNA binding while U_pU_pG does not, and in fact produces binding of Leu-*t*RNA".

Processing of nuclear messenger RNA precursors (pre-mRNA) involves two important steps: the removal of introns — intervening sequences separating the protein coding portions of the gene — by splicing — mechanism selecting which combination of exons contributes to the coding — and the generation of the mature 3' ends by cleavage / polyadenylation. Splicing requires the ordered assembly of large multicomponent complexes, the spliceosomes (Darnell, 1985).

Prebiotic molecules of short RNAs were first carriers of genetic information. This information was encoded in RNA and transferred by synthesis of a complementary replica strand, with the original strand as a template along which complementary nucleotides are assembled according to the base-pairing rules. As proposed by Eigen *et al.* (1981), in prebiotic translation the first code had to establish the direction of readoff and the punctuation of the message by defining a "reading frame". According to Crick *et al.* (1976), directionality and framing were initially fixed by translating only triplets that had a special sequence. The first, primordial protein was made of four amino acids that had a negative electric charge and "which would not readily associate with a negatively charged RNA species unless specific forces stabilized a particular interaction" (Eigen *et al.,* 1981).

In similar fashion to the pairing of the two strands of the DNA double helix two stretches of RNA can form a helical region if they contain complementary nucleotides. By pairing with one another, conserved sequences might determine that the intron folds up into a particular shape. Cech and Bass (1986) have shown, in the protozoan *Tetrahymena,* that this shape helps the RNA to remove the intron portion of its own length which could function as "ribozyme".

Double-strand RNA which is the only genetic information of many groups of viruses (retroviruses, etc.) can be considered as homologous to DNA by reference to the inverse polarity of its two strands. In double stranded RNA viruses, it is also the minus strand which acts as a template for the synthesis of a plus strand acting as mRNA. Retroviruses contain two copies of the plus stranded viral RNA genome. A single RNA molecule may be sufficient for the generation of a complete viral DNA

copy during reverse transcription by strand transfer from the 5' to the 3' end of the same template. There are, however, alternatives to this mechanism (Panganiban and Fiore, 1988).

In the process of translation of mRNAs, proteins are synthesized from their N-terminus to their C-terminus by translating their mRNA polarly from the 5' end of the RNA toward the 3' end. Thus, the 5' half of an mRNA molecule codes for the N-terminal half of a protein and the 3' half codes for the C-terminal half.

Initiation of protein synthesis in eukaryotes requires over a dozen proteins. One of its first steps is the recognition of the cap structure at the 5' end of mRNA by the preinitiation complex. This complex then moves on the mRNA in the 3' direction until an AUG codon is encountered. There, initiation factors are released, elongation factors and the large ribosomal subunit join and protein synthesis begins.

RNA that contains the complementary sequence to a given RNA is termed antisense RNA. The use of this "inverse polarity RNA" permitted to produce "mutants without mutations" (North, 1985). In such mimic mutations the expression of the gene is blocked by preventing the translation of its sense transcripts and phenocopies of mutants are produced (Herskowitz, 1987).

2. Proteins

There is a strong tendency for the polar side chains of proteins to seek a polar environment such as water and for the nonpolar ones to be segregated in nonpolar areas. Except in cell membranes, a protein chain will therefore tend to fold so that polar side chains are on the exposed surface and nonpolar ones are inside.

As first shown by Perutz, electrostatic interactions are of considerable importance in protein structure and function, and in a variety of cellular and biochemical processes (Perutz, 1978; Matthew, 1985). The electrostatic attraction between a polar side chain and water is a form of hydrogen bonding, in which a hydrogen atom acts as a bridge between charged oxygen or nitrogen atoms. In the secondary structure or alpha helix of Linus Pauling, hydrogen bonding between peptide units reduces the energy, but if the helix were unraveled, the same sites would form hydrogen bonds with water. Local interactions between nearby amino acids give rise not only to alpha helices, but also to beta sheets (Pauling, 1960) or other forms of secondary structure. These subassemblies, acting as more or less coherent units, organize themselves into domains of the tertiary structure (Doolittle, 1985). Protein structure is stabilized by interaction of α-helix dipole with a charged side chain. This dipole moment results from the alignment of dipoles of peptide bonds (Fig. 6) which can perturb the pKas of ionizing groups (Săli *et al.*, 1988). Folding of small proteins occurs very rapidly even if it is slowed down by the need for proline isomerization or disulphide crosslinkage. Recently, many proteins have shown to contain Zn^{2+} finger domains

where a short polypeptide could be held in a folded structure by the chelation of Zn^{2+} by two cysteines and two histidines appropriately spaced (Sharp and Eisenberg, 1987).

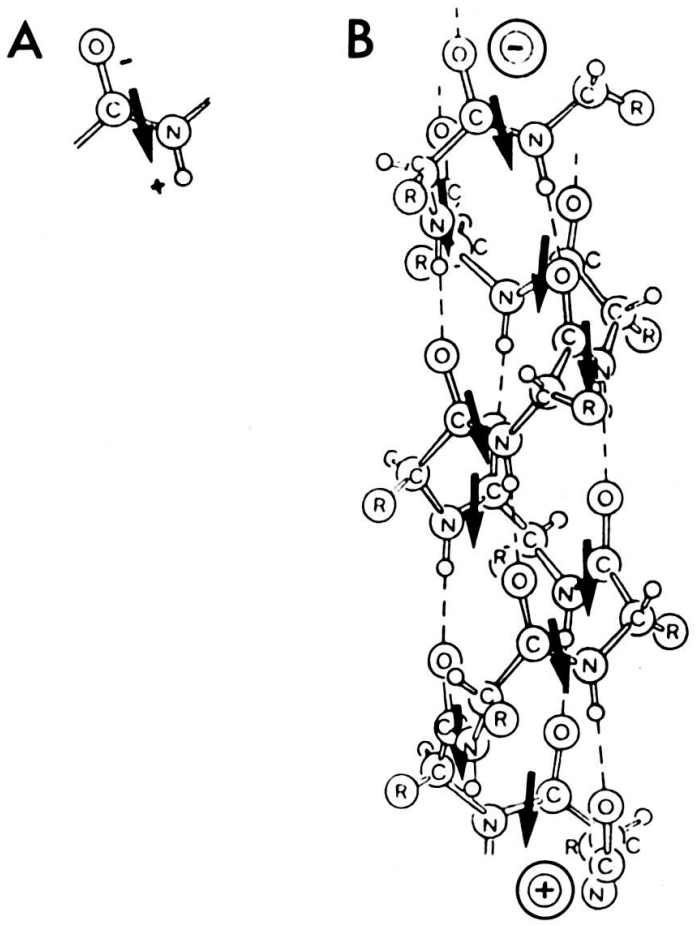


FIG. 6.

(A) Single peptide-bond dipole. (B) Peptide-bond dipoles aligned nearly head to tail within an α-helical protein. Adapted from Creighton, *Nature* 326: 547 (1987).

Recent evidence has shown that the stabilities of helical synthetic polypeptides are influenced by changes in charge at the ends of the helix. Thermostability of a protein such as T4 lysozyme can be increased by introducing charged residues at sites designed to interact with α-helix dipoles. Two mutant lysozymes (Ser Asp; Asn Asp) were designed to increase thermostability by enhanced helix-dipole interactions. An interpretation for each replacement could be that the mutant structure is stabilized by an electrostatic interaction between the negatively-charged side chain and the positive charge attributed to the α-helix dipole (Nicholson *et al.*, 1988).

The peptide-peptide hydrogen bond is the structural element underlying the closely packed helices and beta sheet structure composing the interiors of most

proteins. Net electric dipole moments arise from the uneven distribution of electrons in the carbon-oxygen and nitrogen-hydrogen bonds of each peptide (Matthew, 1985). In the constrained geometry of the protein hydrogen bond network, the peptide dipoles often aligned coherently and parallel to a helix axis may give rise to a macrodipole (Wada, 1976). However, according to Matthew (1985) "it is likely incorrect to uniformly assign the same set of partial charges to peptide dipoles throughout the structures", a problem which should be solved by "an all-atom computation including discrete solvent atoms and individual atomic polarisabilities".

According to Quiocho *et al.* (1987), highly polarized peptide bonds are the net effect of three features involving hydrogen-bonds and the association of peptide units with charged groups which are in turn coupled to hydrogen bond arrays. These authors proposed a general mechanism in which the isolated charges on the various buried, desolvated ionic groups are stabilized by these polarized peptide units.

L-Arabinose-, sulphate- and leucine/isoleucine/valine binding proteins are members of a large group of periplasmic proteins (collectively called "binding proteins") which serve as initial high-affinity receptors of bacterial active transport systems for a variety of nutrients and of sugar chemotaxis (see V). The presence of several helices in the binding-protein structures, together with the concept that the alignment of peptide dipoles parallel to the helix axis may give rise to a macrodipole with partial charges, suggests that helix macrodipoles could provide some means of stabilizing the charges on various ionic groups. Helix macrodipoles could assist in the binding of sulphate, although they have no effect on Arg 151 and the bound leucine. This mechanism could also be applied to the activation of catalytic residues (Quiocho *et al.*, 1987).

At their high level of organization, some proteins can have nonpeptide components such as metal ions required for the activity of certain enzymes or the porphyrin ring found in chlorophyll (Mg) and hemoglobin (Fe). Iron is itself the active center of the group interacting with oxygen. The oxygen-carrying plate-like haem of the globular protein myoglobin has been shown by classical crystallographic studies (Kendrew *et al.*, 1960) to be embedded in a pocket that is lined largely with nonpolar amino acids. However, on the basis of the often minor changes in acid dissociation constants of proteic groups in water and inside certain proteins, Warshel and Russell (1984) have recently deduced that many protein cavities have a high effective polarity despite the lining of nonpolar amino-acid residues. Warshel (1987), in reexamining the theories concerning the interactions of charges located within proteins, points out that the similarity of the dissociation constants of proton-bearing groups within the protein and in water force the conclusion that the protein is polar in their immediate vicinity, and that previous theoretical treatments had neglected the role of peptide bonds in the stabilisation of charges.

The translation of the three dimensional-structure of proteins to electrostatic free energies is far from simple. According to Warshel (1987) "By genetic engineering

techniques, it is now possible to examine theoretical predictions directly by experimentally creating or removing charged residues, and even changing the hydrogen-bonding pattern around such residues''. Modelling of electrostatic effects in proteins — by algorithm — requires consideration of the atomic polarizabilities of the heterogeneous protein and the solvent, including both water and counterions (Warshel and Russell, 1984). The model was founded in classical electrostatic theory, which treats materials as homogeneous dielectric media that may be polarized by electrical charges. Proteins are known to have low dielectrical constants (about 2-3), because reorientation of their dipolar groups is severely restricted. Because water has a very high dielectric constant (± 80), the protein-water interface constitutes a boundary between two dielectric media. The Poisson-Boltzmann equation describes systems composed of charges and dielectric media. To solve it, numerical methods on high-speed computers are implemented (Warshel, 1987). They have already been applied to proteins in studies of substrate diffusion in the electric field of Cu, Zn-superoxide dismutase, the interactions of Klenow fragment of DNA polymerase with B-DNA and the effect of a specific charge on the redox properties of cytochrome C_{551}. As recently put by Warshel (1987) ''It seems clear that only models that take into account both the polarity of the water and of the protein could reproduce the observed energetics''.

Preceding authors (in Warshel, 1987) have attributed electrostatic effects to the macrodipoles of the protein helices (which are in fact almost completely shielded by large dielectric effects) rather than to local polarity. However, isolated ionized groups have been found in protein interiors, for example the aspartate 102 in chymotrypsin (Warshel and Russell, 1984). The recent work by Quiocho *et al.* (1987) in which were identified highly refined cases of uncompensated charges stabilized by the surrounding protein dipoles (positive groups by the C = O dipoles and negative groups by the N-H dipoles) has confirmed this study and thus the importance of the local dipolar components (Warshel, 1987).

The true polarity of the interior of proteins has further been confirmed by the use of bound fluorophores, the spectroscopic properties of which reflect the polarity of their environment. Polar chromophores and fluorophores are molecules that exhibit large differences in the electronic distribution of the ground and fluorescent states. The local polarity of a biological system harbouring such molecules can be estimated by comparison of the spectral properties in that environment with those of the probe in solvents of known polar characteristics. The ideal polar fluorescent probe should have no sources of charge besides two readily identifiable monopoles, and the dipole moment that they determine should change strength but not direction in the excited state. Most important, the probe should be soluble in a range of solvents that cover the polarity scale, from apolar solvents like cyclohexane to the most polar ones like alcohol or water. Optical spectroscopy studies with fluorescent probes such as amino- naphtalene sulfonate (MacGregor and Weber, 1986) have confirmed that

the myoglobin haem cleft has a polar interior and that polarity can be accounted for by peptide amine dipoles (Cowley, 1986).

Chromoprotein molecules such as those of hemoglobin can be associated into fibers. Mutational abnormalities in these fibers can result in abnormal shape of the cell as that of erythrocytes in the sickle (S) cell anemia (Pauling *et al.*, 1949). The only molecular difference between normal and abnormal (S) hemoglobins is the replacement in position 6 of the two β chains of a polar amino acid, glutamic acid, by a non polar one, valine (Ingram, 1956). In sickle cells, the 7 pairs of strands of hemoglobin (HbS) fibers are polarly oriented. Improved, three-dimensional reconstructions from electron micrographs recently showed 3 pairs in one orientation and 4 in opposite orientation (Rodgers *et al.*, 1987). The alignment and the association of these long double-stranded fibers of opposite polarities could thus concour to the distorted shape of the mutated erythrocytes S cells.

Membrane proteins have a number of charged groups on their surface and polarized bonds between their atoms which give rise to bond dipoles. An electric field can therefore exert conformational forces on their molecular structure. Protein's net electric charge might also control polarized transport of proteins as suggested by Woodruff and Telfer (1980) who microinjected fluorescently labelled proteins with different pKs into either the nurse cell or the oocyte of *Drosophila* and followed their movements across the cytoplasmic bridge. Injected electronegative protein, serum globulin, moved only from nurse cell to oocyte, whereas electropositive lysosome only moved from oocyte to nurse cell. These observations suggest that the protein's charge is the main factor determining the direction of intercellular transport (Nuccitelli, 1983). However, by microinjecting fluorescently labelled acidic and basic proteins into the nurse cells or oocyte of vitellogenic *Drosophila* follicles, Bohrmann and Gutzeit (1987) failed to obtain evidence for charge-dependent migration of these molecules.

Electric current can also influence protein biosynthesis. Using collagen matrices of mammalian fibroblasts, McLeod *et al.* (1987) found a reduced rate of incorporation of proline into extra- and intracellular protein depending of the frequency and amplitude of the applied electric field. In tissues containing cells aligned either parallel or perpendicular to the electric field, this response was dependent on the orientation of the cells relative to the electric direction. It was thus demonstrated that "currents of physiological strength can stimulate alterations in protein biosynthesis and thereby may influence tissue growth, remodelling, and repair".

3. Lipids

Neutral fats or triacylglycerols, the major type of lipid stored in cells, are entirely unpolar.

Glycerophosphatides or phospholipids are mainly represented by derivatives of phosphatidic acid in which a phosphate group replaces one of the fatty acids.

Consequently, they are amphipathic, that is one end of the molecule is strongly hydrophobic (i.e. the end containing the hydrocarbon chains) while the other end is hydrophilic due to the charged nature of the dissociated phosphate group and other substituents.

Amphiphiles are surface-active substances or "surfactants" which tend to accumulate at the water surface where they lead to a strong decrease of the surface tension of the solution. They are organic, lipid-type molecules such as soaps and detergents that consist of a hydrophobic, head group, and an oleophilic, hydrocarbon chain; at ambiant temperature, the hydrophilic head groups form hydrogen bonds with water and they can mix water and oil into stable, homogeneous solutions called "microemulsions". There are two classes of amphiphiles: ionics such as the single-tailed sodium dodecyl sulfate (SDS) or the more oleophilic double-tailed compounds; nonionic (uncharged) amphiphiles are not equally well soluble in water and oil (Kahlweit, 1988). At low temperatures, such amphiphiles are more soluble in water than in oil while the reverse is true at elevated temperatures. Nonionic amphiphilic molecules can thus be viewed as "chemical dipoles" rather than electric dipoles. The higher their "dipole moment", that is, the stronger their amphiphilicity, the stronger the tendency of their micelles to form more complex structures such as hexagonal and then lamellar liquid crystals (Kahlweit, 1988).

Of special significance are special membrane structures formed by phospholipids in aqueous solution such as spherical micelles, with hydrophobic interior of fatty acyl side chains, sheets of phospholipids in a bilayer and spherical liposomes comprising one phospholipid bilayer.

Compounds such as steroids and fats, i.e. compounds that have a preponderance of hydrocarbon groupings, which makes them only slightly soluble in water, also have the ability to spread on surfaces and to have a particular orientation so that their polar groups will, in general, be in contact with water or with other polar groups, and those parts that have hydrocarbon groups will be out of contact with the water. Furthermore, lipoprotein films which combine both these two types of compounds, can even be formed artificially, for instance cholesterol and the protein gliadin have been used to produce such a film.

In membranes of fungal hyphae, additionally to phospholipids, there are sphingolipids which are also polar molecules containing hydrophilic "head" and a long hydrophobic "tail" (Weete, 1974). Phosphatidylcholine and phosphatidylethanolamine are the most common phospholipids with phosphatidylserine and phosphatidylinositol present in smaller amounts.

Glycophospholipids are also amphipathic molecules which can anchor proteins to the plasma membrane at the cell surface. A molecule with a bulky polar head group such as glycosylphosphatidylinositol might serve as surface-oriented lipid protein anchor (Low and Saltiel, 1988).

4. Polysaccharides

Homopolysaccharides contain only one type of sugar residue. Oligomers or polymers of uronic acids known as uronides are constituents of pectic substances. Owing to the presence of carboxyl groups, they are polar compounds capable of forming salts (insoluble Ca-pectates). Homopolysaccharides can also become polar by phosphorylation: phosphomannans secreted by yeasts of the genus *Hansenula* have phosphodiester linkages between their mannose residues (Rose, 1968). In cellulose, the successive links of β-glucose are rotated through 180° with respect to each other, but in starch the α-glucose residues can react with each other without rotation. The cellulose chains have a twofold screw axis as an element of symmetry; consequently, they are more stable than the starch molecules which are deprived of axial symmetry (Frey-Wyssling and Mühlethaler, 1965). According to structure analysis, the cellulose chains in the crystal lattice run anti-parallel with respect to each other, so that at the end of a polar fibril the glucose residues must be added in two different ways to form 1,4-bonds with half of the chains, and 4,1-bonds with the remaining chains (Frey-Wyssling and Mühlethaler, 1965).

Heteropolysaccharides contain more than one type of sugar monomers. The capsular heteropolysaccharide responsible for the serological specificity of type III of pneumococci is negatively charged by the carboxyl group of its glucopyranosyl-glucosyl unit. Such polar property of the heteropolysaccharide might intervene in the ionic bonding suggested to be largely responsible for "trapping" the capsular layer around the cell wall (Rose, 1968).

Acid mucopolysaccharides such as hyaluronic acid (alternate group units of glucuronic acid and acetyl-glucosamine), chondroitin, and keratan sulfates have also polar characteristics which might be related to their role in the cells.

5. Enzymes

Enzyme processes can be described by energy surface. The reaction's activation energy is a difference in height of the points on the energy surface representing the starting materials and the transition state. Enzymes can lower the reaction's energy barrier by binding most strongly not to the reactants but the transition state which is thereby stabilized (Pauling, 1960). Hydrolysis of an ester passes through an unstable state whose distinctive shape and charge can be mimicked by a stable molecule. The transition state is tetrahedral and its polarized partial negative charge is concentrated at one apex (Fig. 7). It was recently found that crossed asymmetric insertion — by fusion of encoding DNA — of polar domains of the two aspartate and ornithine transcarbamoylases (AT- and OTCases) from *E. coli* can switch substrate specificity (Houghton *et al.*, 1989).

From his study of the kinetics and specific inhibition of acetylcholine esterase, Wilson (1954) has proposed a bipolar interpretation of the interaction between active

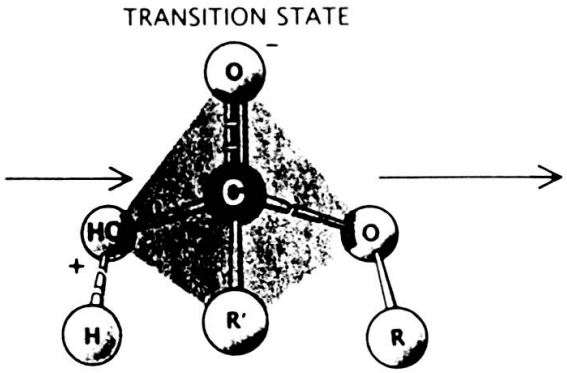


FIG. 7.

Polarized tetrahedral transition state in the hydrolytic process of an ester. (R and R' = chemical groups not taking part in the reaction). From R. A. Lerner and A. Tramontano, Scientific American, vol. 258 (3), p. 46 (1988), with authorization.

groups of the enzyme and its substrate occurring at two sites: the "esterasic site" in which a basic group G donates an electron pair becomes (+) and binds the acyl-group (RCO-) while liberating choline (R'OH); the "anionic group", responsible for the specificity of the enzyme in which a negatively charged group on the enzyme is thought to combine with the positively charged trimethylammonium group of the substrate. This hypothesis of the intermediate formation of acyl-enzymes has received support from several other enzymes among which chymotrypsin. Kinetic studies of several of these enzymes that act at ester and amide bonds have suggested that the imidazolyl group of a histidine residue may serve as the basic electron donor at the positive "esterasic site". In some enzymatic reactions, both general acid and general base are involved as catalysts in concerted proton tranfers. In such polarized reactions, the general acid donates a proton to the transition-state species and the general base accepts one (Lehninger, 1975; Koshland, 1987).

The decarboxylation of β-keto acids such as dimethyl-oxaloacetic acid is catalyzed by metal ions such as Cu^{2+}; this has been attributed to the formation of a chelated intermediate and to the attraction by the (+) charged copper ion of electron away from the β-carboxyl group, with the liberation of CO_2 (Steinberger and Westheimer, 1951). This mechanism may simulate the enzymatic decarboxylation of β-keto acids, which is known to be dependent on the presence of metal ions.

Most of the enzymes catalyzing biosynthetic reactions of phospholipids are amphipathic molecules (Darnell *et al.*, 1986); thus, the introduction of a double bond in stearoyl-CoA to form the CoA derivative of oleic acid is catalyzed by stearoyl-CoA desaturase which has the hydrophilic part of its active site facing the cytosol and the hydrophobic part associated with the endoplasmic reticulum.

Heme-containing enzymes are versatile biocatalysts. They have the ability to incorporate oxygen atoms from O_2 into organic substrates (the oxygenase activity) and to use H_2O_2 and other peroxides to oxidize substrates (the peroxidase activity).

Oxygenases and peroxidases use O_2 and H_2O_2 respectively, through variations in the nature of the proximal axial ligand, in the polarity of the proximal and distal heme environments, and in the degree of accessibility of the heme iron center and the heme edge (Dawson, 1988). The mono-oxygenase cytochrome P-450 has a thiolate axial ligand and a very nonpolar environment for the heme and for the organic-O_2 substrates. The horseradish peroxidase has an imidazole axial ligand and a charged and polar heme environment to facilitate cleavage of the peroxide O-O bond. As for the chlorperoxidase, which can carry out one-electron (peroxidase) as well as two-electron (peroxygenase) reactions, it has a mechanistic activity falling between that of P-450 and that of horseradish peroxidase. The electrostatic potential in and around the active site channel of superoxide dismutase (SOD) has been calculated and its deep channel found to be positively charged, with residues critically placed to guide an incoming negatively charged ion towards the copper ion in the active site (Getzoff *et al.*, 1983). The metalloenzyme SOD which catalyzes the dismutation of O_2- thus provides a particularly good example of an asymmetric distribution of charged groups on the surface of a protein as also occurring in the dipolar cytochrome c (Margoliash *et al.*, 1982) and ribonuclease T1 (Heinemann and Saenger, 1982).

The internal motions that underly the workings of enzymes are best explored in computer simulations (Karplus and McCammon, 1986). These simulations applied to ribonuclease have shown that a number of amino acids in these enzymes make a network of hydrogen bonds to the substrate. In its absence, "water molecules occupy the positions of the polar substrate atoms in the binding sites and produce a network of hydrogen bonds that mimic the substrate interactions and stabilize the positions of binding-site amino acids".

6. ANTIGENS-ANTIBODIES

In the immune reaction, antigen-antibody reactions involve a specific molecular union between the two reagents. Antigens are normally macromolecules such as proteins. Antibodies are specific not only for a given antigen molecule but for certain regions of that antigen, the antigenic determinants or haptens. Hydrophobic haptenic groups tend to form more stable complexes with antibody than do water-soluble groups. Not only hydrophobicity but also polarity have been suggested to play a role in the interaction between antibody and the antigenic determinant (Levy *et al.*, 1973).

Every individual antibody molecule has one type of light (L) chain and one type of heavy (H) chain. The chains are held together by disulfide (-S-S-) bonds to form a monomer; two monomers are linked by -S-S- bonds to form the basic dimeric structure of the antibody. Within each chain, units made up of small peptides of about 110 amino acids fold up to form compact domains. The N-terminal domain is called the variable region and the C-terminal domain is called the constant region.

The variable domains of L and H chains are bound to one another and interact closely to form a single compact unit which is the antibody-binding site to antigen. The three amino acid segments of the variable region contribute to that antigen binding; they are loops that extend from the so-called immunoglobulin fold (Darnell *et al.*, 1986). The binding site being complementary in structure to the antigen, its three segments are therefore called complementarity-determining regions (CDRs). In the CDR regions, amino acids contact the hapten phosphorylcholine and form a cleft or pocket into which the phosphorylcholine hapten fits. A combination of electrostatic, hydrogen-bonding and van der Waals forces hold the hapten in the cleft (Capra and Edmundson, 1977).

The antigen-combining site of antibody molecules consists of six separate loops supported by a conserved β-sheet framework. Modelling of these polypeptide loops with special regard to antibody-binding site modelling are promising X-rays crystallographic technics to allow selection of better-quality antigenic conformations (Bruccoleri *et al.*, 1988). In such model constructions there should be used distance-dependent dielectric constant in the evaluation of electrostatic interactions and given consideration to polarity relationships between backbones of the framework residues in the antigen combining sites.

7. Synthetic polymers

Polymer scientists have designed molecular chains to increase their mutual attractions and thermoplastic's toughness. In pure polystyrene, which consists of a backbone of covalently bonded carbon atoms surrounded by hydrogen atoms and bearing a six-carbon benzene ring on every second backbone atom, the molecule is nonpolar: it lacks localized concentrations of positive and negative charges, which could give rise to electrostatic forces among the chains.

If more polar monomers-monomers with a less symmetric distribution of charges are placed along the polymer chain, electrostatic attraction will contribute to the intermolecular forces. For example, the nonpolar styrene monomers can be interspersed with more polar acrylonitriles monomers, a step that increases the cohesion of the molecules and hence the solvent resistance of the bulk material. The copolymerization of styrene and acrylonitrile is now routine, and many other commercial polymers similarly incorporate two or more different monomer building blocks.

Formaldehyde (CH_2O) polymers can be synthesized in the deep cold at liquid helium temperatures (4.2°K) by a quantum-mechanical effect known as tunnelling which allows some forbidden reactions to take place near absolute zero. Tunnelling results of the particle-wave duality of all matter and radiation and could thus also dominate the behaviour of "particles" such as atoms and molecules (Goldanskii, 1986). The quantum-tunnelling of particles would provide the additional activation

energy to the reacting molecules, an effect also shown to play an important role in redox reactions at low temperature.

A salient feature of polymers is their alternating single and double bonds, which create an electronic configuration that is susceptible to doping. It has recently been found that doping of ordinary polymers by either chemical or electrochemical processes can change their electronic profile of insulators and semi-conductors (Kaner and MacDiarmid, 1988). Electrons are removed from the valence band (positive doping by an iodine solution, for example), or added to the conduction band (negative with a solution of sodium metal in mercury, for example). The charge conferred on a polymer backbone by doping causes a slight but important change in the position of its atoms which prompts the formation of one of three types of charge "islands" called solitons, polarons and bipolarons. Electrons can freely flow between these adjoining islands and so, solitons, polarons and bipolarons are responsible for making polymers conduct. This is well examplified by plastics such as the positively-doped polyacetylene chain which has a delocalized positive charge that enables it to conduct like a metal (Kaner and MacDiarmid, 1988). These recent findings open the interesting possibility of artificial nerves made of polarly conducting polymers.

B. Aggregates

1. Crystals-quasicrystals

A conventional crystal is a particularly well-ordered arrangement of atoms or molecules. It is a lattice-work, in which identical "unit cells" fit together regularly and periodically to fill space. Inherent in every crystal structure are certain symmetries: three- four- sixfold rotational symmetry for equilateral triangle, square, hexagon respectively. The fivefold symmetry is displayed only by quasicrystals which embody a novel kind of order between crystalline and amorphous (Nelson, 1986).

In ionic lattices of crystals, oppositely charged ions exert a polarizing effect upon each other which can lead to deformations of the electron orbits (Fajans, 1925); then the ions can no longer be regarded as spherical and ionic lattices often possess a low symmetry. The ionic lattice is broken down and the solid crystal dissolved when ionic bonds are broken by polar liquids (water, ammonia) and by hydrogen or hydroxyl ions.

Antifreeze polypeptides are aligned at the surface of growing ice crystals. Dipole interactions between the α-helical polypeptide molecules and water molecules in the crystal lattice were found to be maximized for antiparallel alignment (Yang *et al.*, 1988). Adsorption of polar additives will be unfavoured on the basal and prism faces which are the ones with the lower energy. According to the mechanism proposed for the antifreeze effect, the relative orientations of the ice dipoles and the prism faces direct the binding of the protein. The ordering of the ice dipoles by the helices on the surfaces would make these sites less favourable for further growth of the ice crystals.

2. VIRAL ASSEMBLIES

Viruses are built from many protein subunits which are shape determining. Nucleic acids are dispensable in this process as demonstrated by assembly of nucleic acid free viral shells (capsids). Therefore, the ability to determine the shape of the assembly product or aggregate can only be explained by the binding properties and geometry of these protein subunits (Kellenberger, 1984).

a) *Spatial symmetry*

As assemblies of fully shape determining subunits viruses can be considered as the first level of complexity in biological form. One of the simplest is the Tobacco Mosaic Virus (TMV) with its filamentous capsid built as a *cylindrical* assembly of identical protein subunits.

Spherical virions can also be built from at least 60 identical subunits when assuming icosahedral symmetry and the principle of quasi-equivalence for the positions of each protein subunit with respect to each other of the same virus (Caspar and Klug, 1962). Building of larger, closed particles related to icosahedral symmetry rather obey to a quasi-relevance principle, the shape of the proteins being slightly different according to their positioning on the faces, edges or vertices of the icosahedron (Caspar, 1980). The symmetry is therefore only approximately fulfilled. According to Kellenberger (1984), "close contact between subunits is essential, ascertaining the mechanically required, relatively strong interactions. The interactions are the sum of so-called weak (non covalent) interactions that occur in the area of contact".

A very significant feature of organized structures built by use of the specificity of the relatively weak non covalent interactions that are possible between macromolecules (proteins, etc.) is that their design and stability can be determined completely by the bonding properties of their constituent units. Thus, once the component parts are made they "assemble themselves" (Caspar and Klug, 1962) without a template or other specific external control, although, in the case of certain enzymes and in the sheath of the T4 bacteriophage tails, a controlling function may be exerted by the binding of some other small molecule(s); this changes the configuration of the protein molecules so that they have the appropriate bonding properties.

As explained by Klug (1969), the possible types of spatial symmetry are restricted by the geometry of space. Since biological structures are built of molecules which are different from their mirror image, mirror or inversion symmetry is not possible at the molecular level of organization. The only kinds of spatial symmetry operations possible are rotations and translations.

b) *Polar elongative assembly*

The length of a helical structure such as TMV is not determined by symmetry since the structure can repeat indefinitely along a line (the helix axis). A helical arrangement of subunits is therefore theoretically infinite in extent, but in the case of TMV the length of the particle is determined by the length of the RNA.

Electric bipolarization intervenes in the interaction of RNA and protein in the best known of the self-assembly systems, that of the single-stranded TMV: the negative phosphates in the RNA chain are held in place by salt bridges to the positive side chains of two arginines (Arg-90 and Arg-92) of the protein (Klug *et al.*, 1979).

Phage tails are also suitable model-systems for exploring length determination (King *et al.*, 1978; Hohn and Katsura, 1977). In their monopolar assembly lengthening, the central tube or core plays an important role. It has been suggested that "a single gene product is producing a fibrous protein which determines the length by acting as a ruler. The protein would be attached to the base plate and guides the assembly of the subunits around it. The end of the fiber would be the signal for termination of the polymerization" (Kellenberger, 1984).

P66 is a most interesting morphopoietic factor: indeed, if its encoding gene 66 only is mutated, but all other genes active, then appears a highly increased amount of the shortheaded, isometric variant of T4 (references in Kellenberger, 1966). Therefore, this gene product has an "elongating" morphopoietic function.

c) *Polar viral morphopoiesis*

The active process of viral form determination is very precisely genetically predetermined. Contrarily to standard morphogenesis, it is not under time-control of transcription or translation but the consequence of protein-protein, possibly protein-nucleic acid interactions. Therefore, proteins of a precursor particle must first reach a certain conformation to provide binding sites for an additional protein.

The form of bacteriophage pre- or prohead (DNA-filled when mature) has been shown to be determined in a large part by the scaffolding action of their proteineous cores (Kellenberger, 1980); such "scaffolding", "assembly" or "morphopoietic" cores are transient structures which carry form-determining information. The core of the bacteriophage T_4 is especially rich in such information and thus, by the action of drugs or by mutations could be diverted from its normally slightly elongating, so-called prolate icosahedral form to many form variants: short or isometric heads, head length variants and tubular forms, the so-called polyheads produced in the absence of core proteins. In extreme cases, giant tubular phages with tails at both extremities are even produced; this should imply a surprising and still unexplained inversion of assembly polarity or "neobipolarity".

Interactions of electric charges can control the length of phage particles as recently shown by Hunter *et al.* (1987) in the filamentous bacteriophage fd. This

phage comprises a circular, single-stranded DNA molecule enclosed in a cylindrical protein sheath to form a flexible particle. Its coat protein subunit is 50 amino-acid residues in length and, in the virus particle, adopts a largely α-helical conformation, with the long axis of the helix aligned close to the long axis of the filament. This protein is arranged with its negatively charged N-terminal region on the outside of the filament and its positively charged C-terminal region on the inside abutting the DNA. The positive charge on one of the four lysine side chains in the latter region has a direct effect on DNA packaging, because when this charge is absent, elongated particles are produced with lengths that can be correlated with the residual positive charge in the C-terminal region of the coat protein subunit. There are four lysine residues in the C-terminal region of the major coat protein and, according to the same authors, "it is reasonable to suppose that some or all of these have an important function in neutralizing the negatively charged phosphodiester links in the encapsidated DNA".

IV. SUBCELLULAR POLARIZATIONS

A. Protoplasm and cytosol

1. From the protoplasm to the cytoplasm

Schleip (1929) has pointed out that in every attempt to explain the polarity and symmetry of eggs "some as yet unknown property of the protoplasm has to be introduced". He refers to this property as the intimate structure of the protoplasm. According to Seifriz (1935, 1936, 1938), there is in protoplasm a continuity of structure consisting of elongated molecules (as in carbohydrates and proteins) to which the polarity and symmetry of organisms can be attributed. Needham (1942) envisages the protein chains of the protoplasmic lattice as being connected at many points and as being capable of springing back into position after disarrangement of their dynamic paracrystalline state. Harrison (1936), too, has advanced a molecular hypothesis to account for specific regional localizations: the embryonic developmental pattern is to be ascribed to the protein pattern. Because of the bipolar character of protein molecules, Harrison considers that they may become oriented in the cell and, in relation to the different chemical properties at the poles, two material gradients will be initiated. Local reactions will also take place at points along these gradients and the complications of development will ensue. In 1941, Child still stated "protoplasm in general has not yet been shown to possess any structure that might serve as a basis for developmental pattern".

The viscid protoplasm of eggs has thus a long time been considered as apparently very fluid, flowing like a liquid. Such a physical state corresponds to what is now called cytosol. However, with its thixotropic properties it is interconvertible with a cytogel state.

2. Cytosol

The cytosol is the part of the cytoplasm, the cytologically so-called hyaloplasm, remaining after organelles, membranes and ribosomes are removed by centrifugation. This cellular compartment is segregated from the other organelles by a lipid bilayer or unit membrane (Robertson, 1959). In it, protein synthesis takes place on 80S eukaryotic ribosomes and mediates communication between the nucleus and organelles and among the organelles themselves.

All intermediary metabolism takes place in the cytoplasm, most of it in the cytosol. The cytosol generally represents about 55% of the total cell volume and it has a high protein concentration (20-30%). It is teaming with thousands of enzymes

that catalyze the reactions of glycolysis and gluconeogenesis, as well as the biosynthesis of sugars, fatty acids, nucleotides, and amino acids.

It has currently been assumed that the enzymes are dissolved in a homogeneous "soup" of metabolites as suggested by nuclear magnetic resonance experiments. However, the spatio-temporal organization of metabolism would implicate metabolic channelling from producing- to consuming enzymes adsorbed on structural proteins. These would be constituents of an ultra fine microtrabecular lattice ramifying throughout the cytosol (Porter and Bonneville, 1973). Evidence exists that suggests that a number of enzymes of intermediary metabolism including those of glycolysis are bound to the lattice. These enzymes would be associated with the lattice in an ordered (polar?) fashion, successive reactions of the pathway being spatially coordinated (Sheeler and Bianchi, 1987). Water would be free to move the short distance inbetween the threads of this cytomatrix and much of it could be tightly bound to the polar residues on the surface of structural proteins and other macromolecules, as so-called water of hydration. From these new views, the cell sap would emerge as a "bouillon" with "vermicelli", these last corresponding to the lattice of structural proteins (Westerhoff, 1985). In addition, the cytosol contains a variety of different cytoskeletal proteins that impart shape to a cell and cause coherent cytoplasmic movements. With such a complex proteinaceous framework it can then be expected that the cytosol is polar in its organization, especially at the level of the channelling of the enzymes adsorbed on the lattice, more precisely in the "dynamic aspect" of this channelling where metabolite transfer is contingent on — possibly oriented (polar) — collision of the enzymes.

Cytosolic polarities are also suggested from certain cytological studies having shown that the cytosol immediately surrounding the Golgi apparatus is not identical to that closely encircling the cell nucleus. "However, because this type of organization is very difficult to preserve once cells are broken open, we know almost nothing about the mechanisms by which it is achieved" (Alberts *et al.*, 1983).

In higher plants, gradients of cytosol free Ca^{2+} appear to be correlated with polar cell development (Reiss and Herth, 1978); such cytosolic gradients may be related to electric current circuits in developing organs (Jaffe, 1979). The cytosol is also polarly organized in algae (Smith and Grierson, 1982).

B. Surface membranes

1. Primitive membranes

The primeval organic bioreactions need to be catalyzed on large surface systems. Mineral membranes have soon been considered as possible chemical microreactors in such prebiotic syntheses. The alumino-silicate sheets of clays such as mont-

morillonite, smectite, allophane, etc. were first assumed to be important for the origin of life by Bernal (1967). Since, many experiments have used clays as catalysts for the formation of biomolecules such as porphyrins.

In his genetic takeover scenario Cairns-Smith (1982) holds that clays could very well have served as "low-tech" precursors to the self-replicating biomolecules as we now know them. His case for clay membranes for the first photosynthetic apparatus is founded on the hypothesis that first genes and first organic synthetic apparatus would have been made from clays whose asymmetric structures and their naturally membranous character are adapted to that primordial task.

Clay surfaces, with the accompanying counterions in the electrical double layer, are the equivalent of a cathode immersed in an electrolytic solution, except for the continuous electric current. Hence redox reactions could occur readily on such surfaces, provided that energy be made available, for example from reorganization of the clay lattice with an overall decrease in the potential energy. Mineral membranes could hold transition metal cations such as iron atoms to catch light and conduct charge (Anderson and Stucki, 1979) — as well as acting as inert barriers to separate photoproducts. Therefore, in the design of primitive energy transducers, and most particularly for phototransducers where excited photoproducts must be quickly chemically separated, asymmetric mineral membranes were most called for. There had then to be proteins — evolved into modern thylakoid membranes (see IV.D.3) — which took over the primeval clays, to lay out the charge conduits, to hold the right metal ions in the right places, and to maintain the essential asymmetry... "Without proteins how could all this be arranged in an organic membrane? How could genetic information have set up?" (Cairns-Smith, 1982).

In the early stages of earth's development, the formation of proteins, nucleic acids, etc., was not in itself enough to produce living organisms; there had to be a "passage" to a closed organism implicating separation of one part of the continuum from another, the living from the non-living (Bernal, 1967). Groups of primeval molecules responding to polarizing electrical and surface forming forces had to become oriented in a way to produce membranes and vesicles sequestering mineral and organic molecules. Primitive, surface metabolists were therefore faced with desorption of their polyanionic constituents from anisotropically structured, positively-charged mineral surfaces (e.g., pyrite FeS_2) at the interface of hot water (Wächtershäuser, 1988. The secret of the complex functioning of living cells was thus achieved by the isolation, partial or timed, of their various activities by various membranes: the nuclear membrane guards the activity of the nucleus, the Golgi apparatus, the endoplasmic reticulum and the mitochondria have membranes that permit them to carry out activities in partial but not complete seclusion, and the cell or plasma membrane encloses the whole cell.

2. Plasma membrane

a) *Biochemical properties*

The plasma membrane is composed mostly of lipids and proteins in roughly equal amounts; in addition, small quantities of carbohydrates and, occasionally, even nucleic acids have been reported as being present.

Langmuir was the first, in 1917, to deduce from his studies of molecular films spread on water surfaces that lipids at an interface of air and water arrange themselves in a monolayer with the polar ends of the molecule directed toward the water interface and the nonpolar ends at the air interface. In 1925, Gorter and Grendel suggested that the lipid was arranged in the membrane of red cells as bimolecular leaflet with the hydrophobic carbon chains directed toward each other in the interior of the membrane. In the pauci-molecular model proposed in 1943 by Davson and Danielli, the cell membrane consists of a double-layer of lipid molecules covered by two layers of proteins. The nonpolar, hydrophobic groups of lipid molecules face each other as in the bimolecular model of Gorter and Grendel. In 1953, Danielli suggested that the plasma membrane also contains a series of so-called pores that he called polar pores. As groups of protein molecules are oriented radially with their polar groups directed toward the interior of the pore, such molecules would be able to control the penetration of substances through this pore according to their polar group affinities.

In electron microscopy, the apparently solid plasma membrane appeared to be composed of two dense lines representing inner and outer protein layers and the space in the center the double lipid layer. This structure characteristic of all biomembranes was described by J.D. Robertson (1959) as "unit membrane". Thermodynamic requirements implicating maximization of hydrophilic/hydrophobic interaction, however, placed restrictions on this classical model of a trilaminar arrangement of a continuous lipid layer sandwiched between two monolayers of protein and led Singer (1971) to propose his fluid mosaic model. Free energy terms such as hydrophilic-hydrophobic interactions and, secondarily, other noncovalent interactions such as hydrogen bonding and electrostatic interaction were shown to contribute to determine macromolecular structure. These combined effects intervene in the phospholipid bilayer: non polar fatty acid chains sequestered together away from contact with water (hydrophilicity), ionic and zwitterionic groups in direct contact with the aqueous phase (hydrophobicity); dipole-dipole interactions between ion pairs at the surface of the bilayer of zwitterionic phosphatidylcholine might also contribute to membrane stabilization.

Of the three major classes of membrane components — proteins, lipids, and oligosaccharides — the proteins are predominant and their structural properties are therefore of first importance. Singer (1971) discriminated two categories of proteins bound to membranes, which he termed peripheral and integral proteins. Peripheral

proteins are held to the membrane only by rather weak noncovalent interactions and are not associated strongly with membrane lipids. The integral or intrinsic proteins — proteins that traverse the phospholipid bilayer — constitute the major fraction of membrane proteins and would be the only critical for the structural integrity of membranes. The low α-helix content of their native structures has suggested that, in intact membranes, they are largely globular in shape rather than spread out as monolayers. As such, they are a heterogeneous set of globular molecules, each arranged in an amphipathic structure, that is, with the ionic and highly polar groups protruding from the membrane into the aqueous phase, and the nonpolar groups largely buried in the hydrophobic interior of the membrane (Singer and Nicolson, 1972).

The hydrophobic effect is one of the organizing forces in the largely thermodynamically-controlled process of membrane assembly. However, the spontaneous folding of proteins does not only involve hydrophobic forces but also direct polar bonds which contribute to the rigidity of the structure (Tanford, 1978). Steric constraints, hydrogen bonds, and hydration energy of charged groups are equally important. The hydrophobic domain of an otherwise hydrophilic protein might extend into the membrane interior and thus "anchor" the protein (in a non-site-bound fashion) to the surface of the membrane (Katchalski-Katzir *et al.*, 1985). The affinity of ligands held to polar surfaces by specific hydrogen-bonding of dipole interactions might be sufficiently delocalized to permit effective diffusion-driven sliding of the protein over the membrane surface (Berg and von Hippel, 1985).

Amphiphiles are surface-active substances or "surfactants" which tend to accumulate at the water surface where they lead to a strong decrease of the surface tension of the solution. They are organic molecules such as soaps and detergents that consist of a hydrophobic, head group, and an oleophilic, hydrocarbon chain; at ambiant temperature, the hydrophilic head groups form hydrogen bonds with water and they can mix water and oil into stable, homogeneous solutions called "microemulsions". There are two classes of amphiphiles: ionics such as the single-tailed sodium dodecyl sulfate (SDS) or the more oleophilic double-tailed compounds; nonionic (uncharged) amphiphiles are not equally well soluble in water and oil (Kahlweit, 1988). At low temperatures, such amphiphiles are more soluble in water than in oil while the reverse is true at elevated temperatures. Nonionic amphiphilic molecules can thus be viewed as "chemical dipoles" rather than electric dipoles. The higher their "dipole moment", that is, the stronger their amphiphilicity, the stronger the tendency of their micelles to form more complex structures such as hexagonal and then lamellar liquid crystals (Kahlweit, 1988).

The major portion of the phospholipids is in bilayer form in a variety of intact membranes; however, this bilayer might be continuous or interrupted. In the lipid-globular protein mosaic model of membrane structure of Singer (1971), the phospholipids are arranged as a discontinuous bilayer with their ionic and polar heads

in contact with water. With a new technique attaching fluorescent tags to synthetic lipid analogues, Pagano's group (1986) has shown that some lipid classes are restricted to the outer half of the plasma membrane and that transmembrane lipid asymmetry plays a role in cell's elaborate molecular network designed for shuttling membrane — proteins and lipids — components.

According to Singer and Nicolson (1972), the fluid mosaic structure is analogous to a two-dimensional oriented solution of integral proteins (or lipoproteins) in the viscous phospholipid bilayer solvent. As a consequence of this model, the exposed surface of most functional membranes is a mosaic-like structure consisting of a smooth matrix of lipids interrupted by a large number of protein particles. There could then occur free diffusion and intermixing of phospholipids and the proteins within the fluid lipid matrix. Certain of the larger integral proteins could span the entire thickness of the membrane (Fig. 8) and some of the membrane lipid, by strongly binding to them, might thus be structurally differentiated. A new class of membrane proteins, defined as amphitropic proteins, could interact with the bilayer and with putative transmembrane receptor proteins, starting from their soluble, cytosolic form. These cytosolic amphitropic cytoskeletal proteins can exist in a soluble form in the cytosol, and alternatively insert directly into lipid bilayers within the hydrophobic domain (Niggli and Burger, 1987). The recent discovery of specific phospholipid flippase proteins might help to resolve the problem of how lipids with hydrophilic, often charged, polar head groups cross bilayers (Bishop and Bell, 1988).

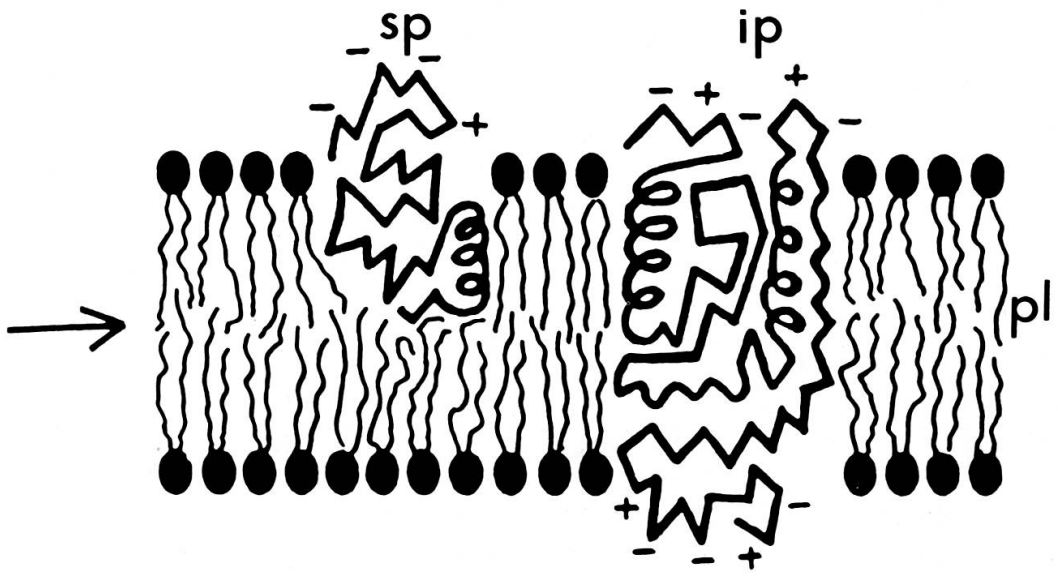


FIG. 8.

Mosaic model of a membrane structure: phospholipid (pl)-globular protein arranged as a discontinuous bilayer with their ionic and polar heads in contact with water. Ionic residues (− and +) of surface proteins (sp); integral proteins (ip) spanning the entire membrane thickness. Adapted from Singer and Nicolson, *Science* 175: 720-731 (1972).

To explain many properties and functions of membranes, the bilayer structure originally proposed by Gorter and Grendel is still attractive. The question remains of how can the many classes of polymorphic membrane lipids that preferentially adopt a non-bilayer configuration be accommodated into the model? In that search, the pioneering X-ray diffraction studies of Luzatti *et al.* (1968) have been extended by electron microscope and nuclear magnetic resonance (Cullis and de Kruijff, 1979). Using well-defined lipid systems, they established that every biological membrane contains large amounts of non-bilayer lipids next to bilayer-forming lipids. This lipid polymorphism is molecularly based on the shape-structure concept (de Kruijff, 1987) which relates the dynamic shape of lipid molecules to aggregate. Bilayer-preferring lipids are roughly cylindrical whereas structure-preferring lipids are cone-shaped with the hydrophobic head group at the smaller end of the cone. Such cone-shape lipids can among others allow for transbilayer transport of polar molecules. Consequently, it appears that cells control their overall lipid composition by lipid polymorphism — the unique ability of hydrated lipids to aggregate into different types of structures — rather than by the previous concept of regulatory «fluidity» (de Kruijff, 1987).

Steroids could also play a part in the structure of membranes, as suggested by their disruption by digitonin, a detergent molecule which has a special ability to form a complex with cholesterol. The free hydroxyl group of this amphipathic molecule could bind molecules of phospholipids by associating with the polar end of the lipid chain which it could curl round. The hydrocarbon part of the cholesterol molecule would be bound to the phospholipid by Van der Waals forces. As cholesterol has a high dielectric constant, it might act as an insulating agent (Bourne, 1970).

Carbohydrate-rich extracellular matrices are commonly attached to the outside of the plasma membrane where they form an outer cell-covering called the glycocalyx (Sheeler and Bianchi, 1987). Such structures are made up of an insoluble meshwork of protein and carbohydrate that is laid down by cells and that fills most of the intercellular spaces with different combinations of collagens, proteoglycans, elastin, hyaluronic acid, and various glycoproteins such as fibronectin and laminin. Acid mucopolysaccharides such as hyaluronic acid are large, negatively charged polymers which can bind H_2O and cations to form the gel-like ground substance of connective tissues. In amoeba, some of the mucopolysaccharides of the thick matrix might be outside from the outer 25 Å layer of the plasma membrane and thus provide surface polar groups (hydrophils). Virtually all of the extracellular matrix glycoproteins and collagens that have been identified interact with cells, and much of the control of cellular behavior appears to originate in response to these interactions.

The plasma membrane of protoplasts can be disrupted by the classical treatment with polyethyleneglycol. There are similarities in this chemical effect and that resulting from the physical application of a momentary, direct electric current pulse or *dielectrophoresis*. During their electrofusion, protoplasts are exposed to a high frequency of alternating electric field which generates dipoles within them. Dipolar

attraction between protoplasts results in their orientation into a configuration reminiscent of a pearl chain (Zimmermann, 1982). Application of the electric current pulse creates pores in the aligned membranes and results in fusion of the protoplasts (Zachrisson and Bornman, 1986). The electrofused protoplasts can survive both chemical or physical treatments, resynthesize their cell walls and undergo division.

b) *Structural polarity and recognition systems*

Biological membranes possess a structural polarity determined by the association of their macromolecular components; it is distinct from the environmental polarity simply due to the presence of different phases on opposite faces of the membrane. According to Changeux and Thiéry (1968), a planar membrane can be oriented in two ways: a *transverse* polarity when it is oriented along an axis perpendicular to the plane of the membrane; a *tangential* polarity when its orientation is along one or several axes contained in the plane of the membrane. When they have the fewest symmetry properties in their lattice structure, such membranes have the highest polarity (Changeux, 1969).

In his recently proposed nomenclature for the organization of biomembranes, Möller (1988) has considered transverse and lateral distribution of membrane components as the central feature of the secondary level of organization. He has listed polarized *vs* non polarized cells, among other examples such as the Golgi apparatus, as relying on this characteristic lateral heterogeneity of membranes. Moreover, at this secondary level of organization, vectorial processes across membranes (transport and signal transduction) are required, and a transverse asymmetry is a consequence. Tangential polarity therefore corresponds to the newly described lateral heterogeneity of membranes, which is an important feature of their secondary level of organization. It is a dynamic process resulting from the "fluidity" of the lipid phase and is important in domain formation (Barber, 1982; Kay *et al.*, 1985).

A substantial amount of experimental evidence has accumulated showing that the two surfaces of membranes are not identical in composition or structure. Both proteins and lipids may indeed be unevenly distributed between the two faces of a membrane such as that of thylakoids (Anderson, 1986). Another aspect of this asymmetry is the distribution of oligosaccharides on the two surfaces of membranes. This has been unraveled by the use of plant protein agglutinins called lectins which bind to specific sugar residues or ligands in a "lock-key" relationship. By conjugating several such agglutinins to ferritin, Singer and Nicolson (1972) have been able to visualize the distribution of oligosaccharides on membranes in the electron microscope. For example, the ferritin conjugate of concanavalin A, a protein agglutinin that binds specifically to terminal α-D-glucopyranosyl or α-D-mannopyranosyl residues, attached specifically to the *outer* surface of erythrocyte

membranes and not at all to the *inner* cytoplasmic surface. A similar, completely asymmetric distribution of ferritin conjugates of ricin has been shown on the membranes of rabbit erythrocytes. Inversely, the enzymatic activity of a membrane protein such as the Na,K-dependent and Mg-dependent adenosine triphosphatase of erythrocytes has been exclusively localized to the inner cytoplasmic surfaces according to Singer and Nicolson (1972) who further commented "if the integral proteins (including the glycoproteins) in intact membranes have, like the phospholipids, an amphipathic structure, a large free energy of activation would be required to rotate the ionic and polar regions of the proteins through the hydrophobic interior of the membrane to the other side."

Changes in the properties of the plasma membrane surface can have cytopathological incidences such as the loss of "contact inhibition" of cells transformed to malignancy; this loss has been shown to be closely correlated with a greatly increased capacity for the transformed cells to be agglutinated by several saccharide-binding plant agglutinins as lectins. The normally "cryptic" sites thus become "exposed" either by proteolytic digestion of normal cells or the processes of malignant transformation (Burger, 1969). In the light of the fluid mosaic model of membrane structure, it has been suggested (Singer and Nicolson, 1972) that mild proteolysis would preferentially release glycopeptides and other polar peptides from the most externally exposed integral proteins. The release of some of the more polar structures would make the remaining portions of glycoproteins more hydrophobic. As these more hydrophobic molecules diffused in the membrane, they might then aggregate in the plane of the membrane. The result would be a clustering of the agglutinin-binding sites on the enzyme-treated cell surface, as compared to the normal untreated surface.

By appropriate manipulation of experimental conditions, e.g. antibody treatment, pH changes, etc., individual membrane proteins can be made to precipitate into aggregates, termed patches (Pinto da Silva, 1972). Under extreme conditions such as lectin treatment, all patches may aggregate to one single membrane region to form a cap (Edelman, 1976). An asymmetric distribution of many membrane receptor components is generated or maintained by cells through this phenomenon of capping which results from a rearrangement of the previously dispersed receptors after their binding to ligand. For example, when exposed to a ligand at a relatively low temperature (4° C), receptors remain uniformly distributed around an initially symmetrical cell; however, the receptors begin to aggregate into patches if the uniformly labelled cell is then warmed up (20-37° C).

Rapid capping by receptor-ligand complexes requires energy and extracellular calcium; the caps form on cells which are no longer spherically symmetrical. Cytochalasins (anti-actin compounds) but not antimicrotubules drugs prevent capping. This suggests a pulling of the ligand-receptor complexes by a contractile mechanism involving polarized actin, myosin, etc. into the region of the cap (Fulton,

1984). Such membrane-cytoskeleton interactions are either direct or through other membrane proteins (α-actinin, etc.) at the capping site.

It is by drawing on energy stored in ATP molecules that the cells maneuver their ligands and receptors together into dense patches. Once a certain patch size is reached the associated cytoplasmic actin, myosin and tubulin act as nucleation centres for the growth of cytoplasmic microfilaments and microtubules and this in its turn effectively anchors the patch within the membrane, preventing further movement. Such processes are important in fixing the polar axis of structures such as eggs of *Fucus* (see VI.A.1b[2]) exposed to a light gradient.

Internally, receptors help to direct newly synthesized proteins to their ultimate destination. A large number of receptors can interact with the cell interior through intermediaries called "G proteins". The prototype G protein-linked receptor is the visual pigment rhodopsin. The light-absorbing portion of the rhodopsin molecule (11-*cis*-retinal) binds in a cavity formed by the transmembrane segments of the proteins. When activated by light the rhodopsin protein undergoes a change in its three-dimensional structure that allows it to react with the corresponding G-proteins, thereby triggering the enzymatic and other changes that produce the cell's response.

On the cytoplasmic side of the plasma membrane, the receptors connect the extracellular matrix to the cytoskeleton. Cell-cell or cell-matrix (glycocalyx) interactions are mediated by the integrin receptors constituting a superfamily of transmembrane glycoproteins. By their attachments to actin filaments, integrins could thus serve as transmembrane linkers between extracellular ligands and the cytoskeleton. Adhesion-promoting proteins have been identified, and the integrin family found to include at least as many receptors recognizing these proteins (Ruoslahti and Pierschbacher, 1987). One polypeptide of the chicken integrin complex has been sequenced from c-DNA and no sequence homology has been found between the α and β subunits of any one of this individual integrin. The amino sequences of integrin strongly suggest that both the α and β subunits span the cell membrane because each polypeptide has a segment with a characteristic of a transmembrane domain near its COOH terminus. The receptors among the integrin required divalent cations such as Ca^{2+} or Mg^{2+} to bind to their ligands and Ca^{2+} has been shown to bind to one of the α subunits.

A signal for targeting proteins to the cell-surface membrane has recently been shown to be provided by a newly described membrane protein (Caras *et al.*, 1987), the "decay accelerating factor" (DAF). The fusion of its last amino acids to the carboxyl terminus of a secreted protein would be sufficient to target the fusion protein to the plasma membrane by means of a glycophospholipid anchor. Together, the adhesion proteins and their receptors constitute a versatile recognition system of surface membranes providing cells not only with anchorage and traction for migration, but important signals for polarity. Whether hazard signals such as those regulating cell differentiation and proliferation can also be generated through cell

adhesion receptors will be an important aspect to study (Ruoslahti and Pierschbacher, 1987; Burridge *et al.*, 1988).

Protein translocation is signal peptide-dependent (Walter and Lingappa, 1986). Correct processing of signal peptide occurs with two enzymes recently identified in *E. coli*: the leader peptidase and the lipoprotein signal peptidase. A schematical representation of this protein export founded on the Engelman and Steitz's hair pin model has been recently proposed by Pugsley and Schwartz (1985). In this model, the NH_2-terminal region of the precursor protein is shown associated with the inner surface of the plasma membrane through interactions between negative charges in the membrane and positive charges in the signal peptide.

c) *Energy transduction*

An important principle of physics is that the coupling between phenomenons of different tensorial orders is not allowed without postulating a structural asymmetry of the system (Curie-Prigogine principle); for instance in active transport, the coupling of chemical reaction (scalar) with vectorial flow of matter requires an asymmetry of the membrane. This apparent paradox of how scalar energy forces could result in vectorial solute transport has been solved by the proposal of the chemiosmotic theory which has revolutionized both the understanding and experimental approach in the area of energy transduction in biological membranes. In such membranes containing active enzymes and substrate or product, which can be acidic or basic and liberating hydrogen or hydroxyl ions, a pH gradient should be produced (Katchalski, 1969).

The principle of polarity or asymmetric structure of biomembranes was basic to the formulation by Peter Mitchell (1961, 1966) of the original chemiosmotic theory which is elegant in the simplicity of its three basic tenets (Johnson, 1987): first, a topologically closed insulating membrane exists with a low permeability to ions and solutes. Second, an anisotopically oriented H^+-ATPase within the biological membrane generates an electrochemical proton gradient. Third, proton-linked antiport or symport transporter systems within the membrane couple the electrochemical potential to ion or metabolic transport (Fig. 9 A, B).

In mitochondria, the energy for building-up the proton gradient can be provided by either premade mitochondrial ATP (ATPase proton pump) or by the oxidative processes of respiration (redox pump) ejecting out couples of protons through the internal membrane of the mitochondria.

The conjugate redox couples participating in oxidative metabolism:

$$2H^+ + 2e^- \longrightarrow H_2 \qquad (E_o' = -0.41)$$

$$\tfrac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O \qquad (E_o' = +0.82)$$

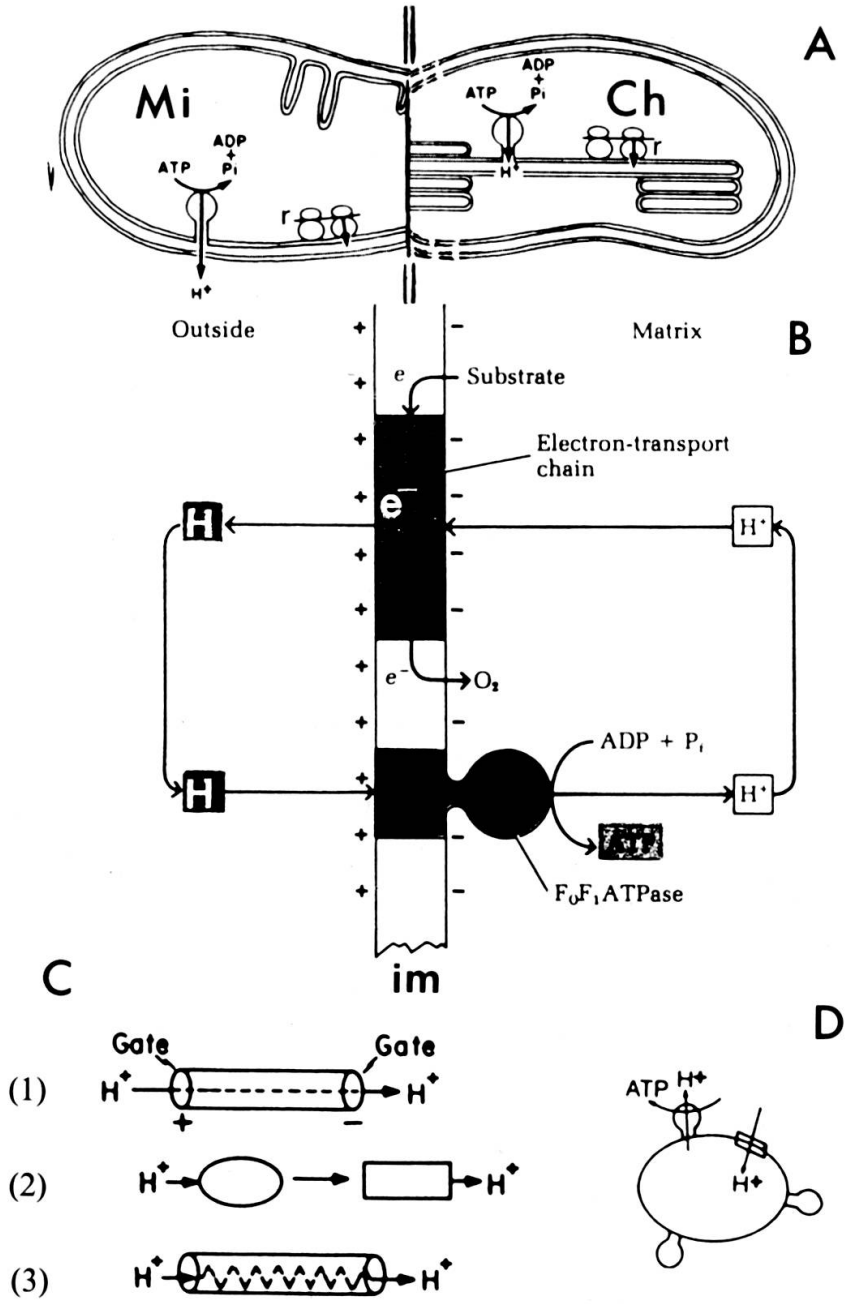


FIG. 9.

(A) Comparative topology of proton gradients and cotranslational insertion into biological membranes of mitochondrion (Mi) and chloroplast (Ch). Note copolar transfer to membrane interspaces of ATPase-pumped protons and of proteins cotranslationally elongated from ribosomes (r). Adapted from Poyton, *Modern Cell Biology* (1983).

(B) Portion of mitochondrial membrane illustrating the principle of the chemiosmotic hypothesis. Inverse polar transport of H^+ (arrows) generating an electric potential across the inner membrane (im) which is positive outside. Adapted from Lehninger, 1982.

(C) Possible models for H^+ translocation in H^+-translocating ATPase: (1) gated channel; (2) conformational change (3) charge relay mechanism. Adapted from P. L. Pedersen, in Transport ATPase, Ann. N.Y. Acad. Sci., vol. 402, 1982.

(D) Inverted mitochondrial vesicle with reverted proton gradient and external ATP synthesis. According to Nicholls, 1982.

occupy the opposite "pole position" of the standard scale of reduction potential (E_o'). The direction of electron flow is always "downhill" toward oxygen. Most of the electron pairs entering the respiratory chain arise from the action of dehydrogenases that use the coenzymes $NAD(P)^+$ as electron acceptors:

$$NAD(P)^+ + H^+ + 2e^- \qquad NAD(P)H \qquad (E_o' = -0.32).$$

Electrons may be transferred from the electron donor to the acceptor in the form of a hydride ion ($:H^-$), which bears two electrons, as in the case of these NAD(P)-linked dehydrogenases (see Lehninger, 1982, p. 256). Pyridine-linked oxidoreductions can also be followed by measurements of changes in pH, since reduction of the coenzymes NAD^+ or $NADP^+$ results in formation of an H^+ ion. Reducing equivalents are transferred as H atoms by some of the electron carriers, such as ubiquinone, and as electrons by others, such as iron sulfur centers and cytochromes. There are large free-energy decreases in the three energy-conserving sites that provide energy for ATP synthesis (Lehninger, 1982, Fig. 19).

According to the fundamental law of bioenergetics (Skulachev, 1981; Nicholls, 1982), the chemical or light energy is used to polarly transport H^+ ions uphill across the hydrophobic barrier of a membrane. The energies of the electrochemical H^+ gradient are transduced to an electrochemical potential difference of hydrogen ions (= protonic potential, $\Delta\mu H$) composed of differences in electric ($\Delta\Psi$) and chemical (ΔpH) potentials. Finally, $\Delta\mu H$ as relatively stable form of energy accumulated is utilized for the ATP synthesis coupled to downhill, inversely H^+ polar movement.

Charge separation occurring across the internal membrane of mitochondria is responsible for the formation of ATP. Mitchell (1966) suggested that its synthesis from ADP + P_i might be due to a reversal of the reaction of an hydrolytic ATPase associated with the mitochondrial internal membrane. The reversal would occur when the ATPase, oriented in a particular way in the membrane (Fig. 9 C), is subjected to the separated H^+ and OH^- ions. Phosphorylation would then be due to the pH shift produced across the membrane thereby reversing to a synthase effect the mitochondrial ATPase activity (Mitchell, 1966). However, the view that any delocalized intermediate, which includes the protonmotive force, could be a major energetic intermediate in electron-transport-linked phosphorylation has recently been questioned by Kell (1987) who "pointed the finger of suspicion at the energetic significance of the putative transmembrane electrical potential".

In summary, the chemiosmotic hypothesis proposes that electron transport in intact mitochondria causes "pumping" of H^+ from their matrix compartment to the outside, to generate the pH gradient across the membrane. The H^+ gradient so formed is energy-rich and causes H^+ to be driven back into the matrix through the F_oF_1 ATPase, thus generating ATP from ADP + P_i. Moreover, according to Lehninger (1982), E. Racker first showed, by 1957, that inverted inner membrane

vesicles (Fig. 9 D), with the F_oF_1 ATPase knobs on the outside surface are also capable of oxidative phosphorylation, thus demonstrating the key role of membrane polarity in ATP synthesis.

Comments by Mitchell (1981) himself provide the best overview of these chemiosmotic concepts of enzyme catalyzed group translocation and specific vectorial ligand conduction which were "introduced with the object of helping to explain the general connection between transport and metabolism in biochemistry, and these concepts were derived by adding a *spatial* dimension to Lipmann's (1946) classical biochemical principle of chemical group potential".

d) *Electric potentials*

An unequal distribution of ions across a biological membrane can give rise to a membrane electric potential. This membrane potential is the voltage difference across a cell's plasma membrane (Alberts *et al.*, 1983). It depends on the distribution of electric charges born from the differential ionic distribution: the concentration of potassium ions inside the cell is about 10 times that in the extracellular fluids, while sodium ions are present in much higher concentrations outside the cell than inside (Darnell *et al.*, 1986). The ionic gradient is generated and maintained by the Na^+K^+-ATPase expending cellular energy. If 2,4-dinitrophenol or another inhibitor of ATP production is added to cells, the membrane potential gradually falls to zero as all the ions equilibrate across the membrane (gradient dissipation).

Virtually all living cells maintain a membrane potential that is negative inside partly because positively charged ions (H^+, Na^+, Ca^{2+}) are preferentially pumped out of the cytoplasm into the external medium. Selective permeability and ionic concentration gradient lead to that difference in electric potential between the inside and the outside of a cell. The voltage potential thus created is a function of the selective and relative permeabilities of the membrane to potassium, sodium, and chloride ions. Cells, of course, contain other ions, such as HPO_4^{2-}, Ca^{2+}, SO_4^{2-}, and Mg^{2+}. However, their membrane permeabilities are very small relative to K^+, Na^+, and Cl^-. Furthermore, in electrically active cells such as nerves and muscles it is only K^+, Na^+, and Cl^- (and occasionally Ca^{2+}) that affect the membrane potential. Increasing the permeability of Na^+ ions depolarizes the membrane; increasing either the K^+ or Cl^- permeability hyperpolarizes it.

Membrane proteins, like all others, have a number of charged groups on their surfaces and polarized bonds (giving rise to bond dipole moments) between their various atoms. The electric field therefore exerts forces on the molecular structure. On the other hand, the internal forces between the parts of a protein molecule are relatively strong and act to make a particular conformation stable against such distorting forces. Thus for many membrane proteins, the effects of changes in the membrane electric field are probably insignificant.

According to Saier and Jacobson (1984) "Theoretical considerations lead to the conclusion that if a transmembrane protein is asymmetrically distributed across a membrane such that the charge distribution at one membrane surface differs from that at the other, the protein will possess a dipole moment. If this dipole moment is not perpendicular to the plane of the membrane or if its magnitude can change upon imposition of an altered potential, the protein will be responsive to the membrane potential. Imposition of an increased transmembrane electrical potential induces the protein to assume a conformation in which the dipole is altered either in magnitude or direction. If this second protein conformation exhibits altered activity relative to the first, transport function will be influenced by the membrane potential".

Ion channels are proteins that participate in the generation and transmission of electrical activity in the nervous system and also in the hormonal regulation of cellular processes (Hille, 1984). They have also been much studied in their relationship with the control of such events as excitation-contraction and stimulus-secretion coupling. In natural ion channels the ion condition pathway is composed of transmembrane helical segment of proteins with aggregated, neutral polar faces. In structural models for the assembled, functional proteins, the polar faces of α-helical segments form neighbouring subunits segregated to form an ion-conducting pore. There are different degrees of polarity at the side chains involved in pore formation. Amphiphilic α-helical segments lining the pore can be either highly charged or involve less polar faces (Guy and Hucho, 1987).

The pentadecapeptide gramicidin A, produced by *Bacillus brevis,* has antibiotic activity arising from its ability to conduct monovalent cations across cell membranes. It is a linear polypeptide of hydrophobic amino acids with alternating L- and D-configurations (opposite chirality). Because of such alternating D-L peptide sequence, gramicidin can make left-handed coils that form hollow cylindrical structures. The central pore is produced by a double helical folding motive because of the stereochemical nature of the amino acids in the gramicidin sequence (Wallace and Ravikumar, 1988). Among ion channels, gramicidin A is the best characterized in terms of its conductance properties and the observed effects of alterations in amino acid side chains on its transport properties (Finkelstein and Andersen, 1981). Gramicidin spontaneously forms dimers which can undergo polymorphic transitions. X-ray studies of the crystal structure of its free form have shown that the molecules are hydrogen-bonded together in the form of a double-stranded antiparallel β-sheet whose strands are in register (Fig. 1, in Salemme, 1988). These antiparallel polypeptidic strands are wrapped into a left-handed helical coil. "All of the hydrogen bonds run nearly parallely to the axis of the helix; half of them point with their dipoles in one direction and half in the opposite direction" (Wallace and Ravikumar, 1988). Gramicidin A has two major conformers designated the "channel" and the "pore" structures which are readily distinguished by their distinctly different circular dichroism spectra and their differential responses to the binding of ions. In the X-ray

crystal structure of the gramicidin-cesium complex the "pore" opening is seen as producing a shift in hydrogen bonding registration between the strands of the coiled and antiparallel sheet (Salemme, 1988). By contrast, the experimental "channel" model is arranged as a head-to-head dimer of left-coiled polypeptide chains (Urry, 1971; Fig. 1D, in Salemme, 1988).

In the recently designed and synthesized ion channel peptide models (Lear *et al.*, 1988), the helices are amphiphilic to provide the desired transmembrane aggregation of polar faces. Leucine was chosen for the apolar face of the helix because of its light hydrophobicity and helix-forming propensity, while serine was chosen for the polar face of the helix because it is polar but uncharged. A sequence repeat of seven residues was chosen to provide registry of the polar and apolar faces in linked heptamers. Interestingly, in one of the peptides synthesized, replacement of one serine per heptad repeat by leucine produced proton-selective channels (Lear *et al.*, 1988). The time between channel opening was found to depend on transmembrane voltage and on the peptide concentration. Increasing the voltage at a given concentration of peptides, increased the frequency of channel opening, indicating that a significant reorientation of electric dipole accompanies channel formation (Honig *et al.*, 1986).

Ion-selective channels can allow the passive movement of ionic species through the membrane, down their concentration gradients. Small inorganic ions which carry the electric charges back and forth across the membrane can traverse the lipid bilayer only by passing through special protein channels which have the characteristics of proteases. When the ion channels open or close, the charge distribution shifts and the membrane potential changes. Neuronal signalling thus depends on channels whose permeability is regulated by the so-called gated channels (Alberts *et al.*, 1983).

Two classes of gated channels are of crucial importance: (1) voltage-gated channels — especially voltage-gated Na^+ channels — play the key role in the explosions of electrical activity by which action potentials are propagated along a nerve cell process; and (2) ligand-gated channels, which convert extracellular chemical signals into electrical signals, play a central role in the operation of synapses. As long as the membrane is depolarized the voltage-gated Na^+ channel cannot reopen. A gating segment of the channel protein is visualized as blocking the channel. A positive amino acid side chain on the surface of the "gate" will create an electric dipole moment. This dipole moment will affect the conformation of the segment of protein because it will tend to orient itself in the electric field to assume a conformation of minimal free energy. In the case of the Na^+ channel this could be the closed state. If however, the membrane is depolarized, the gating molecule moves to realign its dipole moment with the electric field (Fig. 10 A; Alberts *et al.*, 1983).

Because the K^+ channels open a fraction of millisecond or so after the initial depolarization, they are called delay K^+ channels. There is an outward movement of K^+ ions, which tends to repolarize the membrane. In fact, many neurons contain voltage-dependent K^+ channels that transiently open immediately upon depolariza-

tion, increasing the K^+ permeability. They are called immediate K^+ channels oppositely to K^+ channels with delayed rectifier behavior which specialize in repolarizing the cell membrane during the late phase of the action potential (Latorre *et al.*, 1985).

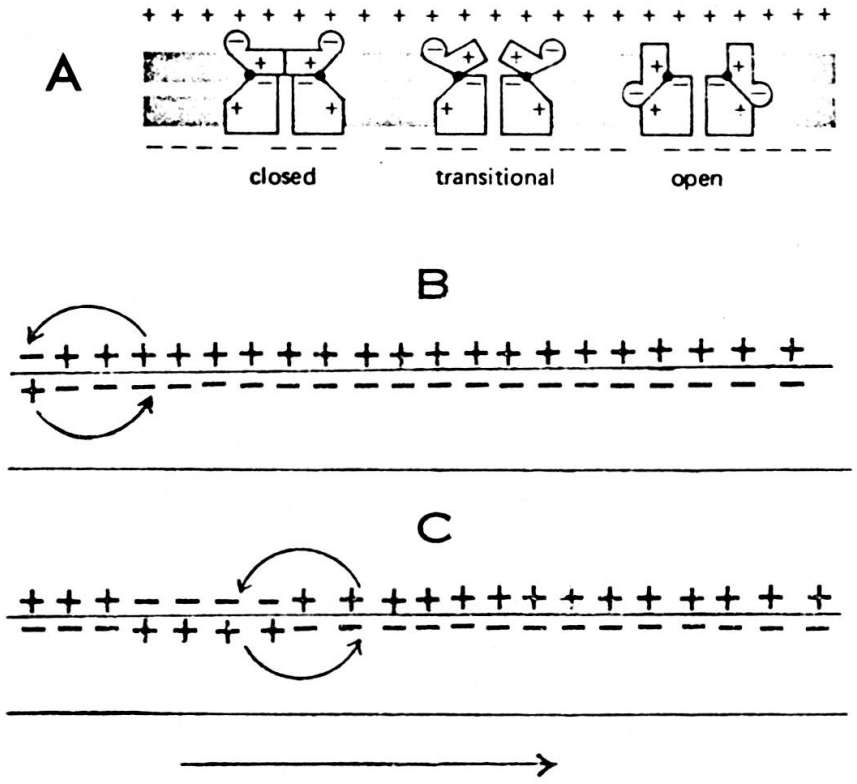


FIG. 10.

(A) Simple model of a voltage-gated channel and its conformational changes during transitions between a closed and an open state. The total energy of a given conformation depends on the membrane potential: when the membrane is strongly polarized, the closed conformation has the lowest energy. Adapted from Alberts *et al.*, 1983.

(B) Action potential in the membrane of an unmyelinated neuron or a muscle fiber excited at one end.

(C) Movement of the potential, followed by recovery. Adapted from Giese, 1979, after Hodgkin, 1957.

Responsive membranes of cells undergo rapid series of *de*polarizations and *re*polarizations. Since the depolarizations result from the opening of a calcium controlled ion channel, the supposition is that the oscillations in membrane potential are brought about by periodic fluctuations in the calcium ion concentrations within the cells. These oscillating concentrations of calcium ions are themselves a direct result of the way in which the ions are released from the endoplasmic reticulum (store of calcium ions) in response to the phospholipase C-mediated hydrolysis of polyphosphoinositide (Berridge, 1987). In the bacterium *Escherichia coli,* depolarization of the cytoplasmic membrane potential has been traced to signal peptide muta-

tions that can cause changes in the level of expression of secreted proteins (Pollitt and Inouye, 1988).

Mechanisms of communication involve interactions at cellular surfaces. Electrical stimuli, passed from one cell to another, may depolarize the receptive cell, thereby causing ion channels within the plasma membrane to open. In many cases, the reception of electrical, chemical or macromolecular signals at the surface of the target cell is transmitted to the nucleus and other cellular organelles, or to intracellular enzymes. In muscle cells, membrane depolarization is transmitted throughout the extensive cytoplasmic system. This electrical stimulation causes Ca^{2+} channels in the sarcoplasmic reticulum to open and release Ca^{2+}, and the increased Ca^{2+} concentration in the cytoplasm brings about the contraction of actomyosin complexes. Consequently, an ion such as calcium can function as a second messenger, transmitting information from a primary messenger at the cell surface by a relay mechanism to intracytoplasmic elements.

In plants, one of the primary events occurring just after light perception by phytochrome involves the transport of ions across membranes, particularly calcium ions (Marmé, 1977). Only the form of the photoreceptor absorbing in the far-red region of the spectrum (P_{fr}) could bind to a specific membrane receptor, resulting in modifications of membrane permeability and membrane potential (Marmé, 1977). Phytochrome bound to membrane undergoes photoisomerization, followed by conformational changes in the proteinaceous part of the holochrome which could lead to a change in the polarization of the membrane. The resulting alterations in the transmembrane potential and the vectorial energy transduction should influence differentially the two isomers of phytochrome, presumably having different dipole moments (Wagner and Cumming, 1970). Phytochrome would thus act as a membrane oscillator, capable of phase shifting rhythmic energy transduction (Wagner, 1977).

At egg fertilization in the sea urchin the first, transient block, is caused by electrical depolarization of the egg plasma membrane, which accompanies the entry of sperm (Jaffe, 1980). This depolarization is caused by ionic changes at the surface (Epel, 1980).

In vision, the initial molecular event occurs in the photoreceptor cells of the retina when 11-*cis* retinal absorbs a photon and is converted into the all-*trans* form (Wald and Hubbard, 1957). At the termination of this excitatory cascade occurring in the outer membrane, the plasma membrane is selectively permeable to ions. Following the illumination, the resulting difference in electric potential between the inside (−) and the outside (+) of the rod cell increases. The increase in potential difference, or hyperpolarization, is due to a decrease in permeability of the membrane to Na ions. This hyperpolarization of the photoreceptor cell membrane has been ascribed to a coupling of photopigments (rhodopsin, etc.) photolysis to cyclic guanosine 5′-phosphate metabolism through a membrane-associated retinal G protein, transducin (Bogenmann *et al.*, 1988).

e) *Action potentials*

Controlled changes in membrane potential are central to the function of electrically active cells. In muscle cells, transient reduction in the membrane potential, or *depolarization* of the surface membrane is often induced by an impulse from a nerve which passes down a neuron and is observed as a movement of negative charges — electrons — along the axon (Fig. 10 B). It is this depolarization which triggers contraction of the muscle (Darnell *et al.*, 1986, pp. 725-726).

The action potential reflects the sequential *de*polarization and *re*polarization of a region of the nerve membrane. It would appear from the outside that negative charges are simply propelled along the outer surface of the neuron, but this process is much more complex. It can be followed by measuring changes in the membrane potential of a very small region of the nerve cell plasma membrane as the electrical impulse passes along it.

Most of the classic studies have been done on the giant axon of the squid, in large part because multiple microelectrodes can be inserted into it without damage and thus the potential across any region of the membrane can be measured. In the resting state, the potential across a small region of the squid axon membrane is -60 mV, typical for most neuronal cells. As the action potential traverses the region, the membrane suddenly becomes depolarized and then the membrane potential becomes about $+35$ mV. The inside is now positive relative to the outside, and there is a net potential change of almost 90 mV. Quite rapidly thereafter, the potential returns to a negative value but to a potential (-75 mV) slightly more negative than the resting value. Gradually the potential returns to the resting value. Thus, the action potential is a cycle of depolarization-hyperpolarization, and return to the resting value (Giese, 1979; Darnell *et al.*, 1986).

The apparent movement of negative charges along the surface of the neuron is, in fact, the successive depolarization-repolarization of adjacent regions of the plasma membrane. The action potential is propagated unidirectionally along the axon, from the axon hillock to the axon terminals. All of these changes in the membrane potential — depolarization, hyperpolarization, and unidirectional propagation — can be ascribed to sodium and potassium channels across the plasma membrane that open and shut in response to stimulation of the membrane. These channels are transmembrane proteins whose ion permeability is dependent on the electric potential across the membrane. According to Serrano's comments (1988), the transmembrane segments of the stalk portion of the Ca^{2+}-ATPase "would constitute a polar channel facilitating the access of the transported cations from the binding sites to the external side of the membrane".

Voltage-dependent Ca^{2+} channels are important for contraction of muscle as well as for triggering the rise of intracellular Ca^{2+} ions that causes exocytosis of synaptic vesicles in the axon terminals. Voltage-dependent ion channels are, in fact,

critical for all aspects of the function of electrically active cells. How then such channels and the coordinate changes in ion permeabilities can explain the action potential? The sudden depolarization of a region of the surface membrane during the action potential is caused by a sudden massive increase in the permeability of that region to Na^+ ions. This increase in Na^+ permeability is caused by the opening of voltage-dependent Na^+ channels. The channels open briefly when the membrane is depolarized — more channels open as the depolarization of the membrane is increased. As more and more Na^+ ions enter the cell, the inside of the cell membrane becomes more positive and the membrane is depolarized further. This depolarization causes the opening of more Na^+ channels. If the initial depolarization of the region of membrane exceeds a certain minimum value called the threshold potential, it sets into motion an explosive entry of Na^+ ions that is completed within a fraction of millisecond. This is the origin of the action potential.

The Na^+ channels of nerve cell membrane selectively allow the passage of Na^+ down its electrochemical gradient from the external medium into the cell, while K^+ channels in the membrane selectively allow passage of this ion down its electrochemical gradient, from regions of high concentrations (inside the cell) to regions of lower concentrations (outside the cell). Appropriate regulation of the activities of these two ion channel complexes can give rise to a sudden, transient change in membrane potential or action potential. Membranes exhibiting the capacity to generate action potentials are said to be excitable.

Neurons, like all other cells, expend a great deal of metabolic energy pumping Na^+ out and K^+ in by means of an Na^+-K^+ ATPase in the plasma membrane. As a result, the concentration of Na^+ is about ten times lower inside the cell than it is outside while the distribution of potassium is roughly the reverse.

How is the resting potential of an excitable cell altered during the development of an action potential? The positive charge on the outer surface of the cell membrane, because of the presence of positively charged ions, is delicately balanced by the negatively charged anions of salts, organic acids, and so on, inside the cell. According to Giese (1979), maintenance of the resting potential of a cell has been found to depend upon the presence of oxygen. Thus, when free oxygen is lacking in the brown alga *Halicystis*, the resting potential falls, but it rises again when oxygen is readmitted or the plant is illuminated (Blinks, 1955; Gaffey and Mullins, 1959). Lack of oxygen reduces the resting potential of the squid giant axon and other neurons, and the reduction is accompanied by a loss of potassium (Hodgkin and Keynes, 1953). Since glycolytic metabolism occurs in the absence of oxygen, the nerve cell continues to liberate energy, but does so at a reduced rate, as indicated by a decrease in liberation of heat.

According to De Robertis *et al.* (1979), nerve conduction is propagated along the axonal membrane by the action potential. This consists of a sudden depolarization with increased permeability to Na^+. The membrane potential may depolarize from

-90 mV and may overshoot to $+50$ mV. In the ascending phase of the spike there is entrance of Na^+. In the descending phase, K^+ leaks out. The action potential has a threshold of activation, is an all-or-none response, is non-decremental, and has a refractory period. As for the time of change in membrane potential, it depends on the membrane capacitance of the axonal electric cable.

That sodium is the conductor of current during the rise of the action potential is demonstrated by substituting for it in the medium bathing the neuron an organic cation, choline chloride, that does not penetrate the cell as readily as Na^+ does. However, the strongest support for the sodium theory of the action potential is provided by controlling the membrane potential and measuring the current flow (voltage clamp experiment). For this a feedback amplifier is connected by two electrodes on either side of the membrane to automatically supply the current needed to shift the membrane potential and maintain it at any desired level.

In the theory of the propagation of the action potential, it is to be expected that local currents will flow from the unstimulated, positively charged areas of the cell membrane to the stimulated, depolarized, or negative portions, and as each new area becomes depolarized or negative, it in turn acts as the *sink* toward which the current flows from the adjacent area of a neuron and serves to give rise to a new regenerative impulse. Progressive depolarization, or reversal of the charge, of the entire length of the neuron therefore follows from the point of stimulation outwardly, as if an electrode were travelling along the nerve fiber (Brazier, 1968). It has been demonstrated that the source of the current is the flow of Na^+ into the cell. This, briefly, is the so-called local circuit theory of propagation of the action potential.

The propagation of the nerve impulse is generally explained by this so-called local circuit theory: at the point of stimulation the area becomes depolarized (negative outside) and acts as a sink toward which the current flows from the adjacent areas. This wave of depolarization advances along the nerve fiber at the rate of conduction that is characteristic for each fiber. While this wave of depolarization advances, repolarization is so rapid that only a fraction of the nerve fiber is depolarized at a time.

In the recovery period, sodium leaves the cell by the action of the sodium pump and potassium re-enters to restore the steady state. This recovery is probably produced at the expense of high energy phosphate bonds. However, impulses continue to discharge for some time in the absence of oxygen, and even when glycolysis is inhibited, which indicates that high energy bonds are stored at the membrane (Darnell *et al.*, 1986).

Opening of K^+ channels tends to repolarize the membrane and hinder the generation of an action potential. If the number of open Na^+ channels and inrushing Na^+ are insufficient to depolarize the cell to the threshold value, no action potential will be generated. The generation of an action potential is thus said to be all-or-nothing.

Electrical activity in *non*-neuronal cells can be induced by altering the membrane potential and eliciting action potentials. For example, hormones, nutrients and neurotransmitters act on excitable endocrine cells. Schlegel *et al.* (1987) showed that a single action potential leads to marked transient increase in cytosolic free calcium. The size of these short-lived maxima is sufficient to evoke secretory activity.

f) *Synaptic membranes*

Synaptic function is related to the processes mediating the control of muscle function by motor nerves, which involves the release of acetylcholine at the neuromuscular junction. There is a rich variety of synaptic transmissions in the central nervous system (Poggio and Koch, 1987) implying both *pre*synaptic mechanisms regulating neurotransmitter storage and release and properties of voltage-gated ion channels at work in *post*synaptic cells. Typical polarized synapses comprise the great majority in the nervous system of both vertebrates and invertebrates; however, a more modern definition of the synapse should also include the existence of a complex submicroscopic organization in both the pre- and postsynaptic parts of the junction and of the specific neurochemical mechanism in which transmitter, receptor protein, synthetic and hydrolytic enzymes, and so forth, are involved (Darnell *et al.*, 1986).

Understanding the generation of electrical signals in neurons requires some understanding of the events that precede them. As we have said, each branching dendrite of a neuron carries many hundreds of synapses receiving signals from "presynaptic" cells. When the action potential of a presynaptic cell arrives at the synapse, it triggers the release of a chemical messenger into the synapse. This messenger, called a neurotransmitter, diffuses across the small space between cells and binds to specialized receptors in the "postsynaptic" dendritic membrane. Neurotransmitter binding induces the opening of specialized channels in the cell membrane. The open channels allow ions, molecules or atoms that bear an electric charge, to enter and leave the nerve cell. This opening and shutting of ion channels in the neuron membrane governs the electrical status of the cell. The most important ions taking part in this process are sodium, potassium and chloride. Pumps in the cell membrane increase the concentrations of certain ions and expel others; hence there is more potassium inside a cell than there is outside, and more sodium and chloride in the fluid surrounding the cell. The differences in concentration constitute what is called a concentration gradient for each ion.

Some synapses transmit excitation and some transmit inhibition, depending on which ion channels they regulate. When ion channels are open, the direction in which each ion moves is determined by the forces exerted by concentration and potential gradients. Hence sodium, a positively charged ion, diffuses into the cell through open sodium channels because both its concentration gradient and its potential gradient favor that direction. The influx of positive sodium ions causes depolarization of the

nerve cell; therefore synapses that control sodium channels are excitatory. By the same principle, potassium, which also bears a positive charge, leaves the cell when potassium channels are open.

In the aspect of synaptic transmission called depolarization-release coupling, one neuron (the presynaptic cell) releases a biologically active substance (a neurotransmitter), which evokes a response, either excitatory or inhibitory, in a second neuron (the postsynaptic cell). The release of the transmitter is in essence a process of secretion, a process shared by cells throughout the evolutionary sequence. In virtually every known instance secretion is accomplished by vesicular exocytosis (see IV.C.3).

Purified synaptic vesicles were found to undergo unidirected transport when injected into squid giant axons (Schroer *et al.*, 1985). In that case, vesicles should bind and activate only one polarity-specific motor at a time. This specificity may be mediated by a receptor on the organelle membrane to which a motility protein could be bound (Vale, 1987). Such a protein might be synapsin I found to interact with synaptic vesicles in nerve terminals (Huttner *et al.*, 1983). A major integral protein of synaptic vesicle, synaptophysin shows a novel cytoplasmic domain and four transmembrane regions (Südhof *et al.*, 1987). As hexameric channel protein, it would be implicated in vesicular uptake and release of secretory compounds (Thomas *et al.*, 1988).

Biochemical study of neurotransmitters and their receptors (see Johnson, 1987) has been focused on such topical areas as the coexistence of neurotransmitters and neuropeptides, synergism between multiple chemical messengers, the role of protein phosphorylation in slow postsynaptic responses and the molecular analysis of the γ-aminobutyric acid (GABA)/benzodiazepine receptor.

As a consequence of ions distribution and the contributions of other charged molecules in the cell, the inside of a neuron is more negative than the outside by about 60 to 90 millivolts (a millivolt is equal to one-thousandth of a volt; a 1.5-volt battery will power a flash-light). This voltage is known as the resting potential because it is present when the cell is not conducting an impulse. The difference in voltage between the inside and the outside of the cell creates what is known as the potential gradient (Poggio and Koch, 1987). When synaptic stimulation opens up ion channels near the synapse, ions enter and leave the cell and the resting potential in this localized area is disturbed. The cell is said to be depolarized if this potential becomes more positive and hyperpolarized if it becomes more negative. This newly generated potential travels toward the cell body, decaying as it propagates, in a manner similar to electrical signal propagation in an underwater cable. If the final depolarization at the cell body is pronounced enough, it will induce an action potential (§e).

In each synapse between brain neurons, when the presynaptic terminal is activated by an action potential, small vesicles fuse with the presynaptic membrane and release a chemical neurotransmitter onto the adjacent postsynaptic membrane.

Ion-conducting channels are open by the receptor-activated transmitter and provoke a brief depolarization of the postsynaptic membrane. Synaptic plasticity is the first step in making accurate network models of memory formation, one form of which, long term potentiation, provides a long-lasting increase in the effectiveness of synaptic transmission. Two of the hypotheses proposed to explain it invoke protein kinases, the first being C-kinase via calcium ions and/or diacylglycerol, the second involving Ca^{2+}/calmodulin-dependent protein kinase (Kaczmarek and Levitan, 1987). A third hypothesis invokes calcium-activated proteases which cleave the cytoskeletal proteins fodrin and microtubule-associated protein (Lynch and Baudry, 1984).

The precise mechanism by which depolarization arises is inconsequential as far as the subsequent release of neurotransmitter is concerned. In neurons such as sensory receptors that respond to stimuli (a burst of sound, a touch on the skin) impinging directly on the surface of the cell, the depolarization is caused by the energy of the stimulus itself. Here the depolarization is typically a rapid modulation in voltage that lasts for only a few milliseconds. Among the neurotransmitters, there are the amino acid γ-aminobutyric acid (GABA) as well peptides such as enkephalins which are flanked with signals (pairs of amino acids). Biogenic amines such as catecholamine, dopamine, norepinephrine, etc. are derived from tyrosine and can also act as hormones. To all these neurotransmitters correspond receptors (see Kennedy, 1988). Depolarization is nonetheless not sufficient in itself to cause the release of transmitter. In addition, a supply of calcium ions must be present in the extracellular environment. Depolarization seems to be the mean by which an inward current of calcium ions is induced to flow through the membrane of the presynaptic terminal. In this respect too, synaptic transmission resembles other triggered secretory processes.

C. Endomembranar and vesicular systems

The cell's membranous components form a temporal and spatial continuum. Rough endoplasmic reticulum is believed to bud from the nuclear membrane; lysosomes and other membrane-bound vesicles form from Golgi complex; plasma membrane in many cells is an important contributor (via pinocytosis) to the formation of the internal cytoplasmic structures.

1. Endoplasmic reticulum

As a network of interconnected, closed membrane vesicles, endoplasmic reticulum (ER) is generally the largest eukaryotic cell membrane. Historically, the ER has been subdivided into two structurally distinct types: rough (RER), which

is studded with ribosomes, and smooth (SER). The outer nuclear membrane contains ribosomes and is continuous with RER (Fawcett, 1981, in Rose and Doms, 1988). The SER is the site of the synthesis and metabolism of fatty acids and phospholipids. The RER, by its bound ribosomes, participates to the synthesis of certain membrane and organelle proteins as well as the proteins secreted from the cell. Oppositely, cytosolic proteins such as actin are synthesized by free ribosomes. A process of protein sorting then targets them to their destination during or after their synthesis.

The ribosomes are formed of many proteins and a few RNA molecules which are held in a characteristic and specific three-dimensional arrangement by many individually weak but collectively strong forces of attraction, such as hydrogen bonds. Ribosomes are asymmetrical structures which must first dissociate into their two oddly shaped subunits each time a new polypeptide chain is begun. They become functional when the larger subunit (50 or 60 S) fits again on the small one (30 or 40 S). In the complete, prokaryotic 70 S initiation complex thus formed, the correct binding of the N-formylmethionyl-$tRNA^{fMeth}$ is assured by two points of recognition and attachment. At translation, the two subunits fit together in such a way that a cleft is formed through which the mRNA passes as the ribosome moves polarly from codon to codon toward its 3′ end, adding an amino acid residue each time (see Lehninger, 1982, pp. 880-890). In an eukaryotic polyribosome, many ribosomes are reading mRNA simultaneously, also polarly moving from its 5′ to its 3′ end, and the polypeptide chains become longer as the ribosomes move toward the 3′ end.

The rough ER membrane is "translocation competent" as is the inner membrane of mitochondria but not the smooth ER or the Golgi complex (Wickner and Lodish, 1985). Signal recognition sequences specify proteins that are targeted to and translocated across the membrane of the ER. They have a wide degree of sequence diversity and the main common denominator appears to be a clustering aggregation of hydrophobic amino acids. The "classical" receptor or signal recognition particule (SRP) shares its burden with another protein, the signal-sequence receptor (SSR), an integral membrane protein of the ER (Wiedmann *et al.*, 1987). SRP and its receptor are involved only in the initiation of transfer of the nascent chain across the membrane, then they dissociate from the ribosome-nascent chain complex and recycle to direct insertion of additional proteins. It should now be further attempted to determine the extent to which other signals are encoded in more complex structures determined by polypeptide folding (Rose and Doms, 1988).

The unidirectional translocation of proteins across membranes is either cotranslational or posttranslational. In the first model, protein synthesis and protein insertion into the ER have to be tightly coupled (Blobel and Sabatini, 1971). However, there are cases such as in yeast in which the ER can translocate finished polypeptide chains. Such a posttranslational mechanism seems evident for small proteins "which are almost completely synthesized before the signal sequence has fully emerged from the ribosome, or for proteins whose targeting signals are the COO^- terminus". It

must, however, be cautioned that recent advances have conduced to blur the simple picture of a clear dichotomy of co- and posttranslational systems (Verner and Schatz, 1988). The amphiphilic signal for targeting proteins across the ER membrane is located in the NH_2-terminal end. Its basic region is followed by stretch of at least 7-8 apolar largely hydrophobic residues (Blobel and Sabatini, 1971). It is a specific "signal pepidase" located on the trans-side of the target membrane which removes the NH2-terminal targeting signal (leader or signal sequence). The removal of the signal peptide is followed by the polar internalization of the growing secretory polypeptide in the lumen, or central cavity of the rough ER from which it moves to the lumenal cavity of another group of membrane-limited organelles, the Golgi vesicles located near the nucleus in many cells. How a secretory protein moves from the lumen of the ER to the lumen of the Golgi vesicles is controversial (Darnell *et al.*, 1986). From the available evidence, it seems that small membranous vesicles containing some of the lumenal ER content bud off from regions of the rough ER not coated with ribosomes and then fuse with the membranous Golgi sacs. Secretory proteins are synthesized in association with the ER membrane but not with plasma membrane. A signal recognition particle must target them there. This single active component — the SRP — was purified from the mixture of proteins stripped from microsomal vesicles (0.5 M NaCl). SRP may have evolved to allow for a coupling between translation and translocation, possibly to allow the superposition of additional controlled mechanisms.

The bulk flow from ER to Golgi to plasma membrane also implies transport of lipids. Many data point toward vesicular transport as a primary mechanism of lipid transport pathways which ensure delivery of cholesterol and sphingolipid to the plasma membrane, delivery of phospholipids to the mitochondria, and retrieval of these types of lipids from the plasma membrane (Bishop and Bell, 1988). There is preliminary evidence for vesicular transport from mitochondria to the ER and it remains to unravel the pathways involved in delivery of phospholipids from the plasma membrane to the mitochondria. "Additional complexities of lipid sorting exist in polarized cells" (Bishop and Bell, 1988).

2. Golgi apparatus

The Golgi apparatus is structurally and biochemically polarized. It is probably the principal director of macromolecular traffic in the cell. It has two distinct faces: a *cis*, or forming face and a *trans*, or maturing face. The *cis* face is closely associated with a smooth transitional portion of the rough ER. In secretory cells, the *trans* face is the face closest to the plasma membrane; here, the large secretory vesicles are found exclusively in association with the *trans* face of a Golgi stack. Proteins are commonly thought to enter a Golgi stack from the ER on the *cis* side and to exit for multiple destinations on the *trans* side. Histochemical tests have been used in conjunction

with electron microscopy to localize particular proteins. They have revealed membrane-bound enzyme activities that show a distinct polarity in their localization within the Golgi stack. Secretory proteins have also been found by histochemical methods in all of the stacked cisternae, even though the large secretory vesicles in which these products are concentrated are associated only with the *trans*-most Golgi cisternae (Fig. 11).

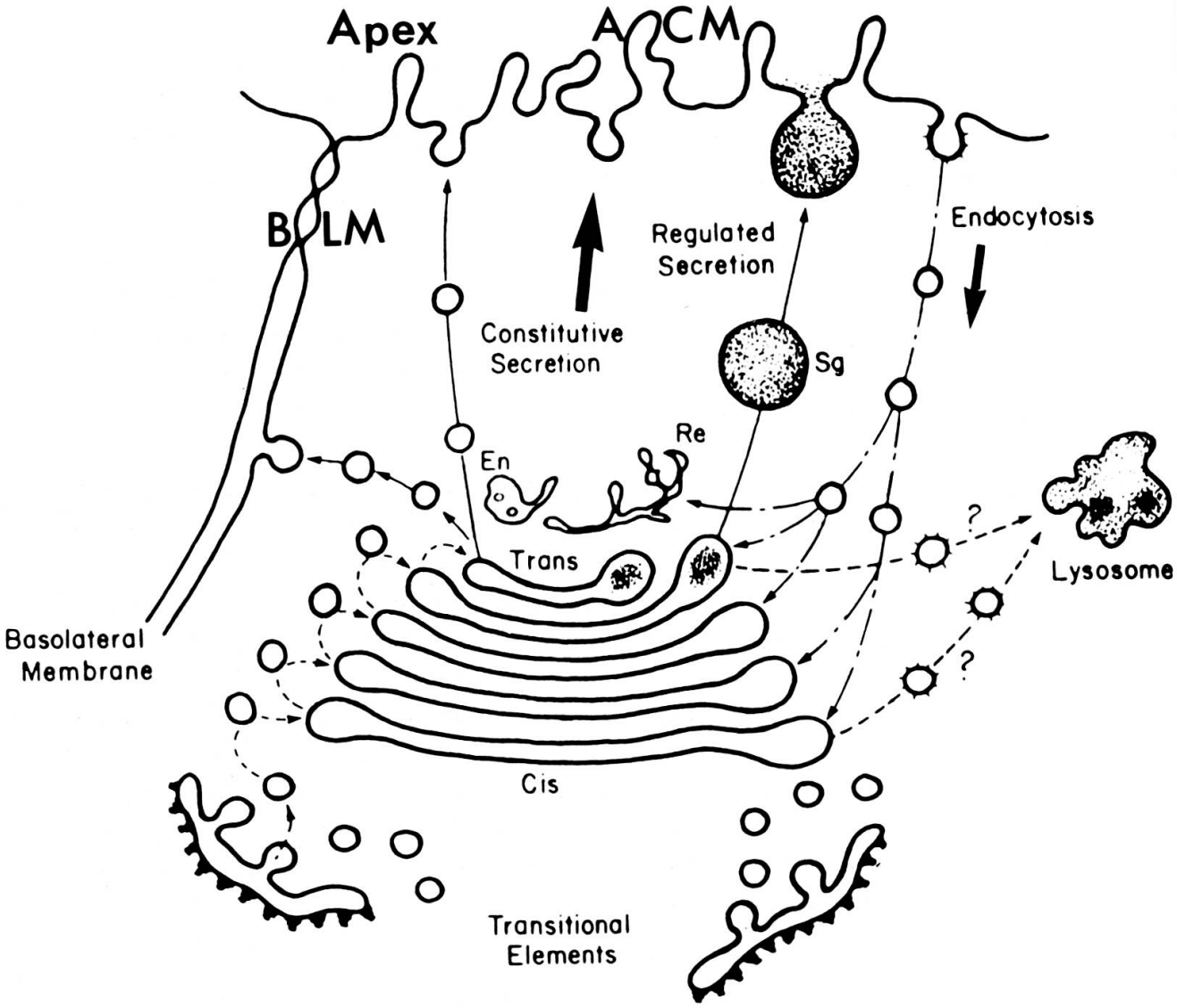


FIG. 11.

Major routes for biosynthetic (---), exocytic (-O-), endocytic (---), and lysosomal (---?) traffic. Only traffic that is directed to or through the Golgi complex is illustrated. The *biosynthetic pathway* from ER to Golgi and across the Golgi stack is utilized for the transport of secretory proteins and membrane glycoproteins. *Lysosomal enzymes* follow the same pathway as secretory proteins from the ER to the *cis* Golgi cisternae. Several different *exocytosis* routes are indicated. *Secretory proteins* can be discharged by either the *regulated* or *constitutive* (non regulated) pathways. *Membrane glycoproteins* are also delivered to the plasmalemma in vesicles discharged by exocytosis. In polarized cells, two separate pathways exist for delivery of membrane proteins to the apical cell membrane (ACM) and basolateral membrane (BLM). Sorting is believed to occur at the time of exit from the Golgi or shortly thereafter. Reproduced from Farquhar, Ann. Rev. Cell Biol. vol. 1 (1985). With the authorization of Annual Reviews Inc., Palo Alto, U.S.A.

Intracellular traffic pathways include: 1) traffic from the rough ER to the Golgi complex, 2) from the Golgi to the plasma membrane or *exo*cytosis, 3) back-traffic from the plasma membrane to the Golgi complex or *endo*cytosis (Fig. 11). Newly synthesized proteins are assumed to be transported unidirectionally from the rough ER to the *cis* Golgi cisternae. This occurred by vesicular transport (see IV.C.3) across the stacked cisternae "in which the enzyme and transport systems responsible for post-translational modifications of proteins are distributed in polarized series" (Farquhar, 1985). Operationally, ER transport out of the ER can be distinguished from Golgi function by its sensitivity to an inhibitor of oxidative phosphorylation, CCCP (Godelaine *et al.*, 1981, in Farquhar, 1985), while intra-Golgi transport can be slowed or arrested by the cation ionophore monensin (Tartakoff and Vassalli, 1977).

Among molecules which can pass through some portion of the Golgi structure at some stage in their maturation, usually shortly after their synthesis in the ER, there are secreted proteins, glycoproteins-proteoglycans, glycolipids, plasma membrane glycoproteins, proteins of lysosomes. Proteins are commonly thought to enter a Golgi stack from the ER on the *cis* side and to exit for multiple destinations on the *trans* side; however, neither their exact path through the Golgi apparatus nor how they travel from cisterna to cisterna along each stack are known. A model has recently been proposed by Griffiths and Simons (1986) whereby these three different classes of proteins are sorted into different vesicles in the last Golgi compartment, the *trans* Golgi network. This compartment corresponds to a tubular reticulum on the *trans* side of the Golgi stack, previously called Golgi endoplasmic reticulum lysosomes (GERL).

Rather direct evidence that protein secretion is polarized in the direction of Golgi polarization is provided by the study of the killing of a target cell by a cytotoxic T lymphocyte: when the granular lymphocyte encounters the target cell there is a rapid reorientation of the Golgi apparatus and microtubule-organizing centre toward the target cell. According to Burgess and Kelly (1987) "the observation that the cytoskeletal protein talin — often associated with sites of microfilament attachment to the membrane — is concentrated by the effector cell (but not by the target cell) at the area of the contact — site of action of the cytolytic pore-forming protein — provides an intriguing clue as to how such polarity may be established in lymphocytes".

Polarity of the Golgi is maintained in part by a process of membrane differentiation, observable as a progressive change in cisternal membrane characteristics from the *cis* or forming face to the *trans* or maturing and secreting face (Grove *et al.*, 1968). Although this entire process has not been demonstrated in every secretory cell (Whaley, 1975), the polarity of the Golgi apparatus is unmistakable in scale-forming organisms as a result of the gradual synthesis of the scales. In the golden flagellate *Pleurochrysis*, membrane flow in the Golgi apparatus is accompanied by synthesis

and assembly of the three scale subcomponents, establishing and maintaining the characteristic dorso-ventrality of the scales in the cell wall (Romanovicz, 1981).

The asymmetric location of the Golgi system has been incriminated in a tentative explanation of the animal-vegetal difference in pigmentation of the cortex of small oocytes during a short period at the start of vitellogenesis (see also VII.C.6a). If the Golgi terminus of the cell were a site of active plasma membrane insertion, then a new pigmentless surface might originate from one pole, while the old and less expanding surface might gradually recede with all the pigment granules (Dumont, 1972).

3. Intracellular vesicles

Golgi vesicles are often referred to as the "traffic police" of the cell (Darnell *et al.*, 1986). They play a key role in sorting many of the cell's proteins and membrane constituents, and in directing them to their proper destinations. To perform this function, the Golgi vesicles contain a number of enzymes that react with and modify secretory proteins passing through the Golgi lumen, or membrane proteins and glycoproteins that are transiently in the Golgi membranes as they are en route to their final destinations. For example, a Golgi enzyme may add a "signal" or "tag", such as a carbohydrate or phosphate residue, to certain proteins to direct them to their proper sites in the cell. Or, a proteolytic enzyme in the Golgi secretory vesicles may cut a secretory or membrane protein into two or more specific segments; this is what occurs in the conversion of proinsulin into insulin.

Still remarkably little is known about the molecular mechanisms involved in driving, guiding and regulating this vesicular traffic. It is clear that these processes require metabolic energy, probably in the form of ATP hydrolysis, but it is not known how the energy is used. Moreover, how do vesicles know where to go? There must be molecules on their cytoplasmic surfaces to guide the vesicles to the appropriate membrane, but nothing is known about their nature or diversity. The continual membrane fusions that occur in vesicular transport might be expected to lead to large-scale membrane intermixing, yet the various membranes within cells maintain their characteristic compositions. Secretory vesicles fuse only with the apical portions of the plasma membrane (see VII.C.6b), thereby avoiding fruitless and dangerous discharges into the spaces between cells or into other organelles in the cell.

The concentrated secretory proteins are stored in secretory vesicles that reside in the apical region of the cell between the Golgi apparatus and the lumen of a secretory duct. The secretory proteins are discharged from the cell by exocytosis, a process in which the secretory vesicles fuse with the plasma membrane to release their contents to the outside. Exocytosis is a highly specific and polarly regulated process. Other proteins such as proinsulin manufactured in the rough ER travel within the Golgi stack from the *cis* to *trans* pole. After the intercisternal movement carried

out by "microvesicles", vesicles reach the *trans* side of the Golgi where they become clathrin-coated and pinch as coated secretory granules. The cleavage process of proinsulin to insulin is accompanied by internal acidification of the then uncoated granules which finally release insulin at the cell membrane (see Fig. in Orci *et al.*, 1988). Organelle acidification in transport and/or targeting is thus an important feature of the secretory pathway as newly synthesized proteins encounter a gradually more acidic environment as they pass through the cell interior (Mellman *et al.*, 1986). As expected, the ionophore monensin wich exchanges a K^+ for a H^+ and so neutralizes intravesicular pH and, consequently, the pH gradient, blocks movement of newly synthesized proteins through the cell (Burgess and Kelly, 1987). Acidotropic drugs can therefore have a dramatic effect on secretion from several cell types.

After secretory proteins are modified in the Golgi vesicles, they are transported out of the complex by membranous secretory vesicles, which seem to bud off the *trans* side of the complex. In many cells, the membranes of these vesicles quickly fuse with the plasma membrane, releasing their contents into the extracellular space. The fusion of the membrane of an intracellular vesicles with the plasma membrane is termed *exocytosis*. In other cells, the Golgi vesicles fuse with secretory vesicles that do not immediately release their contents to the outside. Rather, these secretory vesicles fuse with other similar vesicles and become intracellular membrane-limited storage reservoirs for the secretory proteins. When an appropriate signal, such as a hormone, stimulates a cell, the secretory vesicles fuse with the surface membrane, releasing their products into the extracellular fluid.

There is a strong correlation between the polarity of the Golgi apparatus and the polarity of exocytosis which strongly suggests the association of all vesicles carrying newly synthesized proteins with the microtubular system radiating from the vicinity of the Golgi apparatus. These microtubules all have the same polarity (see also IV.E.3) with the plus ends oriented away from the nucleus. Microtubule-disrupting drugs have provided indirect evidence for this model of polarized transport considered as plausible but not proven on short distances from the Golgi to the plasma membrane; as commented by Burgess and Kelly (1987) "polarization of the Golgi itself may give sufficient polarity to exocytosis and diffusion without kinesin-mediated transport may be sufficient to achieve polarized secretion of proteins".

In the dynamic process called receptor-mediated *endocytosis*, large molecules are brought into the cell through the membrane by a transbilayer "flip-flop" movement of small vesicles. Certain protein receptor molecules are anchored by their tail in the plasma membrane and bind large nutrient molecules which, at this stage are called ligands; many such receptor molecules and their bound nutrients are engulfed in pits developed in the membrane (Goldstein *et al.*, 1985). The pits then close up and bud off into the cell, forming vesicles coated with a lattice of a large, fibrous protein named clathrin. This protein has been isolated and characterized from animal and plant tissues (see Brodsky, 1988) as well as yeast (Payne and Schekman, 1985)

and the filamentous fungus *N. crassa* (Caesar-Ton That *et al.,* 1987). It consists of three arms called triskelions, which assemble to form a cage-like structure by mutual bonding of the arms (Willingham and Pastan, 1984). At the same time other vesicles from the interior of the cell fuse with the plasma membrane. Such bidirectional process of budding and fusing circulates membranes from the surface of the cell to its interior and back again (Schekman, 1985). Moreover, the membrane involved in that dynamic movement during the endocytotic cycle always maintains its topology and asymmetry (Bretscher, 1985).

The function of clathrin-coated vesicles is essentially related to the selective uptake of proteins (Pearse and Bretscher, 1981). On their *exocytotic* path these vesicles surround the *cis* Golgi cisternae and the uncoated cargo is delivered at the docking site. Inversely, during receptor-mediated *endocytosis,* coated pits invaginate to form clathrin-coated vesicles. Clathrin's role, in both inward and outward membrane traffic, would thus be to "facilitate receptor transport by a concentration and sorting process that initiates targeting to specific intracellular compartments" (Brodsky, 1988).

Calcium is involved in exocytosis and endocytosis in plant cells (Steer, 1988). Exocytosis is triggered on cell activation by an intracellular signal which is often a rise in concentration of cytosolic calcium. In some systems, Ca^{2+} inflow is "restricted to a certain part of the plasma membrane, which then acts as a focus for exocytosis and, thereby, establishes a cellular polarity" (Steer, 1988).

In both animal and plant cells, the vesicle membrane is a newly synthesized fragment of plasma membrane, which is presumed to possess docking sites and this could account for vesicle fusions. In freeze-etched images of the plasma membrane (Dahl *et al.,* 1979), docking proteins appear as rosettes of membrane particles.

Many of the important physiological functions of neuroendocrine secretory vesicles, such as the chromaffin granules (Johnson, 1987), are directly related to the existence of an electrochemical proton gradient due to the operation of an anisotopically directed H^+-translocating ATPase within the membrane. This proton pump catalyzes the vectorial movement of protons from the cytosol to the intragranular space resulting in the establishment of a transmembrane pH gradient (ΔpH, inside acidic) and transmembrane electrical gradient ($\Delta\Psi$, inside positive), both contributing to the establishment and magnitude of the electrochemical potential (180 mV). Investigation of the effect of the H^+ gradient upon amine transport has been influenced by documentation of the pivotal role of H^+-ATPase in establishing transmembrane H^+ gradients and their coupling to energy transduction and ion and metabolite transport in a variety of cells and subcellular organelles (Johnson, 1987). The relationship between the $\Delta\mu_{H+}$ and the equilibrium potential for the hormone or neurotransmitter is predicted by the chemiosmotic hypothesis, which states that the energy for the substrate transport system is derived from the $\Delta\mu_{H+}$ generated by the H^+-translocating ATPase (see IV.B.2c).

D. ORGANELLES

1. Lysosomes and peroxisomes

During their transport from ER to lysosomes, the enzymes are first transferred to the Golgi complex in which they are phosphorylated as thought to occur in the *cis* compartment. This phosphorylation serves as address tag that allows the enzyme proteins to bind to a mannose *cis* phosphate receptor. Then those lysosomal enzymes which become sialylated must move through the whole Golgi stack in the mildly acidic *trans* Golgi network (cyto-immunochemical studies of this TGN, see Griffiths and Simons, 1986).

Lysosomal enzymes, mainly hydrolases, are selectively modified in the *cis* cisternae of the Golgi apparatus. In the lumen of the Golgi they are recognized and thought to be sorted into lysosomes. Any protein that lacks a lysosomal "sorting domain" would be transported out of the Golgi to the plasma membrane. According to a first model, the directional choice involves a sorting domain on the secreted protein which must be recognized by an appropriate carrier protein that transports the molecule to the correct secretory organelle (Blobel, 1980). In an alternative view (Kelly, 1985), "the transport of secretory protein in the constitutively secreting cells may involve a passive, bulk flow mechanism in which no sorting is required". This model of dispensability of specific domain on the secreted protein would be supported by the aberrant secretion out of the cell of incorrectly targeted lysosome enzymes.

Peroxisomes (microbodies, glyoxysomes *pro parte*), historically thought to bud from the ER, share at least respiratory (based on H_2O_2 metabolism) and fatty acid oxidative capabilities. Their proteins which are synthesized on free polyribosomes include matrix and crystalloid core proteins, as well as one major integral membrane protein (Lazarow and Fujiki, 1985).

Peroxisomal protein accumulation is slightly different from mitochondrial protein importation. Both processes are posttranslational and the majority of peroxisomal proteins are not synthesized as precursors. The location of targeting information is not yet precisely known (White and Scandalios, 1988). Hydrophilic signals target proteins into peroxisomes without missorting with mitochondria or chloroplasts. A COOH-terminal is the usual targeting signal and only peroxisomal 3-oxoacyl-coenzyme A thiolase has a NH_2-terminal extension (ref. in Verner and Schatz, 1988).

2. Mitochondria

These respiratory organelles are found in the cytoplasm of all aerobic cells, with the exception of bacteria in which the respiratory enzymes are located in the plasma membrane. They are, in general, uniformly distributed throughout the cytoplasm, but there are many exceptions to this rule. In some cases, they accumulate preferen-

tially around the nucleus or in the peripheral cytoplasm. Overloading with inclusions, such as glycogen and fat, displaces these organelles. During mitosis, mitochondria are concentrated near the spindle, and upon division of the cell they are distributed in approximately equal number between the daughter cells. Shape and structure of mitochondria might be metabolically-controlled in situ (Bereiter-Hahn and Vöth, 1983).

The distribution of mitochondria within the cytoplasm should be considered in relation to their function as energy suppliers. In some cells they can move freely, carrying ATP where needed, but in others they are located permanently near the region of the cell where presumably more energy is needed. For example, in certain muscle cells, mitochondria are grouped like rings or braces around the I-band of the myofibril. In the rod and cone cells of the retina all mitochondria are located in a portion of the inner segment.

The location and orientation of mitochondria have already been reviewed by Cowdry in 1918. Electron microscope investigations have confirmed one of his earliest generalizations, namely that in epithelial cells the mitochondria are polarized; their long axes are disposed in the direction of the active secretion or transport process characteristic of that type of cell. Such polarized orientation is beautifully shown in the tubular cells of the kidney (Fig. 12 A), where the mitochondria are aligned in the axis between the blood and the lumen of the tubule (Lehninger, 1965). The basal mitochondria of the kidney tubules are intimately related to the infoldings of the plasma membrane in this region of the cell. It is assumed that this close relationship with the membrane is related to the supply of energy for the transcellular active transport of water and solutes. According to Lehninger (1965): "The orientation of the mitochondria along the axis of active transport across the epithelial cell barrier very strongly suggests that mitochondria are involved in transcellular active transport in a directionally polarized manner. A microscopic gradient of ion concentrations in the hyaloplasm along the length of a single mitochondrion is thus conceivable; along it, the ion-transport function of the mitochondrial membrane may act".

In its resting state a pancreatic cell is also typical in the polarization of its components (De Robertis *et al.*, 1979). The base of the cell is occupied by the nucleus, the basophilic substance containing ribonucleoproteins, and elongate mitochondria oriented in the apicobasal direction. The apical or excretory region is occupied by refractile granules with a high protein concentration. In the supranuclear zone and among the zymogen granules is a Golgi complex.

Mitochondria are also often arranged in helical sheaths surrounding the midpiece of the spermatozoon; presumably, this permits ATP to be utilized for motility of the tail through a very short diffusion path. Similarly, mitochondria are found immediately below the cell membrane near the base of the cilia in protozoa and other ciliated cells; again these are ATP-requiring structures. Mitochondria are also

concentrated near synaptic junctions of axons, where energy exchanges take place during impulse transmission.

The number of crests per unit volume of a mitochondrion is variable. Mitochondria in liver and germinal cells have few crests and an abundant matrix, whereas those in certain muscle cells have numerous crests and little matrix. In some cases the crests are so numerous that they may have a quasi-crystalline disposition. The greatest

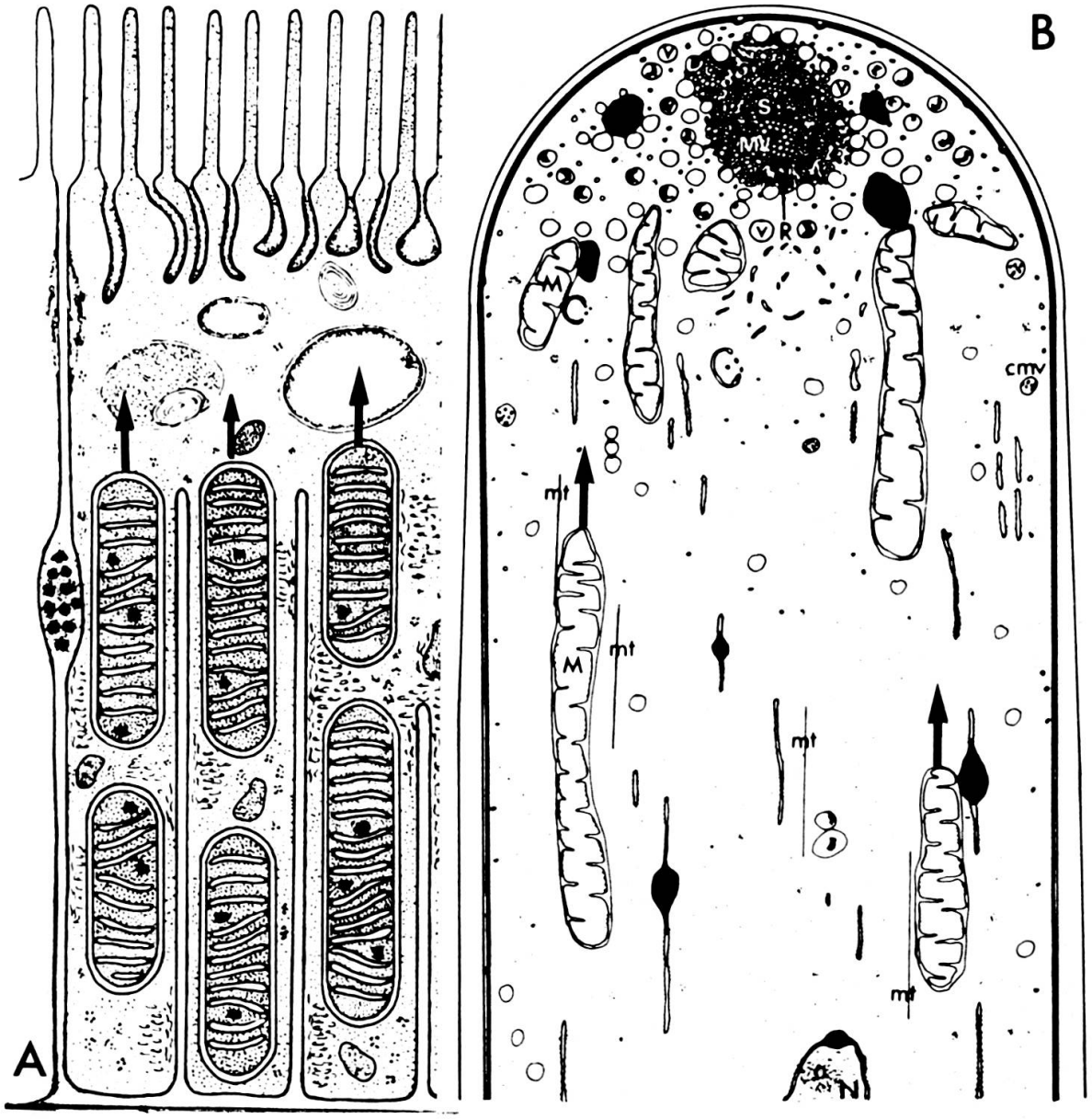


FIG. 12.

Directional polarization of mitochondria (M) in axis of: (A) active transport in proximal renal tubule cells; from Rhodin, in Lehninger (1965). (B) Fungal hyphal tip *(Monilia fructigena)*; mt = microtubule; N = noyau; v = vesicle; s = "Spitzenkörper" (Fig. 23 in Najim and Turian, Can. J. Microbiol. 57: 1299-1313 (1979).

concentration of crests is found in the flight muscle of insects. In general, there seems to be a correlation between the number of crests and the oxidative activity of the mitochondrion.

The energy of mitochondrial respiration is conserved as the electrochemical activity of protons or protonmotive force created by the polar translocation of protons from inside to outside the membrane and driven by a proton pump linked with electron transfer along the respiratory chain. The protonmotive force then drives the ATP pump in the opposite direction with the concomitant synthesis of ATP. The ATP synthesizing machinery is made up of: the F_1 ATPase, a globular protein projecting on the inside of the matrix in intact mitochondria (Fig. 9 A); the hydrophobic F_o (o for oligomycin) embedded in the membrane; some factors (F_c type) necessary for attachment of F_1 to the membrane. In "right-side-out" submitochondrial electron-transport particles (ETPs), the F_1 proteins are projected on the outside and the complex $F_o.F_1$ (F_c included) retains a rudimentary system for oxidative phosphorylation and polarly inversed synthetase activity (see IV.B.2c).

Their high affinity for Ca^{2+} supports a biological role for mitochondria in Ca^{2+} transport. The wide range of Ca concentrations measured in isolated mitochondria and the uncertainty of the effects of isolation has led to a considerable controversy about their Ca content *in situ*: either mitochondria contain sufficient Ca to effectively regulate cytoplasmic free Ca^{2+} via the mitochondrial Ca efflux pathway or mitochondrial Ca is very low and compatible with modulation of Ca-sensitive mitochondrial enzymes through small (micromolar) fluctuations in mitochondrial matrix free Ca^{2+}. The mitochondrion possesses a calcium pump with about the same rate constant as the plasma membrane but more total transporting surface (Borle, 1973; Simkiss, 1974). The polarized pumping membrane is one of the hypotheses proposed to account for transcellular calcium translocation. It would involve the taking up of calcium by the mitochondria from one region and its transport and release at another. Among factors shown to cause mitochondria to release calcium there are pH, Mg^{2+}, Na^+ and K^+ ions or membrane potential. How Ca^{2+} uptake is achieved at one surface of such "vectorial mitochondrion" and release at another remains, however, an untested speculation (Simkiss, 1974). Recent experimental evidence shows that calcium travels across the membrane electrophoretically, pulled by the negative electrical potential maintained inside mitochondria by coupled respiration (Carafoli, 1981). However, uptake and release of Ca^{2+} are separate pathways: calcium leaves mitochondria on a route different from the electrophoretic uptake uniporter which can be blocked by ruthenium red.

Mitochondrial protein import requires targeting signals to specifically route every protein into the organelle's compartments (matrix, inner membrane, intermembrane space, and outer membrane). Hydrophilic signal sequences polarly target proteins into mitochondria. These signal peptides are amphiphilic only if folded into a secondary structure such as α-helix or β-sheet, and have a hydrophobic moment

perpendicular to long axis of these conformations (Schatz, 1987). Usually the signal has the NH_2-terminal as polar domain; even so, the occasional COOH-terminal has been related to the mitochondrial import of apocytochrome c. Unidirectional, polar translocation of proteins across membranes of mitochondria should require an input of energy. It is across their inner membrane that membrane potential ($\Delta\Psi H$ see IV.B.2c) is necessary to move the NH_2-terminal part of a precursor across both mitochondrial membranes; however, it is not required — neither ATP energy — for the subsequent translocation of the entire precursor into the mitochondria (Verner and Schatz, 1988). Matrix targeting sequences can form amphiphilic structures but is not clear how the amphiphilicity of these sequences contributes to their targeting function. It is, however, intriguing that "positively charged amphiphilic peptides such as melittin can penetrate across planar phospholipid bilayers in response to a transmembrane potential of the same polarity (positive outside) as that of mitochondria" (Kempf *et al.*, 1982).

Penetration of the protein precursors (aminoterminal presequence), first bound to receptor like component(s) on the mitochondrial surface requires an electrochemical potential (positive outside) across the inner membrane. The question is whether the immediate source of energy is derived from ATP or from the electrochemical gradient. Proof of the necessity of the electrochemical gradient came from the use of the ionophores valinomycin (K^+ ionophore) and nigericin (H^+/K^+ exchanger) among others. Results tended to show that ATP cannot directly supply the necessary energy (Verner and Schatz, 1988).

Giant mitochondria (networks of thin mitochondrial tubules) discovered in many types of eukaryotic cells (yeasts and protists) have been considered as structures responsible for $\Delta\mu H$ transmission (Skulachev, 1980). Technical difficulties have prevented its direct measurement and it could only be speculated that the inner membrane of giant mitochondria might play the role of electric cables to transmit power in the form of membrane potential. By analogy, an electric potential difference generated across the cytoplasmic membrane near one end of a cyanobacterial trichome was shown to be transmitted along their column of cells functioning as an electric cable and utilized in its distal end to carry work (Skulachev, 1981).

The possibility remains therefore that individual mitochondria have an overall electric polarity: primary and secondary ion transport systems might be localized at one rather than both of their extremities. Such a view of "bipolar mitochondrion" would fit with the proposal that the internal membrane of mitochondria could consist of mosaic domains of differential (high and low) conductance (Azzone *et al.*, 1978). Thus, certain mitochondria longitudinally positioned toward the pole of elongating fungal hyphae might be considered as bipolar, their frontal pole being depolarized (uncoupled) comparatively to their distal pole (Fig. 21; Turian *et al.*, 1985, 1988). In support of the relevance of this differential polarization of mitochondria to monopolar axiation of hyphae, it has been observed that, in germinating conidia

of *Neurospora crassa,* either full depolarization of mitochondrial membranes by uncoupling agents such as the classical protonophore 2,4-dinitrophenol (Pall, 1977) or by valinomycin (Johnson *et al.,* 1981), or their full hyperpolarization by nigericin (Johnson *et al.,* 1981), both treatments preventing electrical *bi*polarity of apico-frontal mitochondria, disturb polar outgrowth of germ tubes (Turian, 1988). Indeed, could such functional bipolarity of mitochondria provide an answer to the pertinent question raised by Attardi and Schatz (1988) of why are these organelles not spherical but elongated?

3. Chloroplasts and phototransducing membranes

In primitive photosynthesis, organic molecules have been suggested by Granick (1957) to be synthesized around impure magnetic particles of pyrite (FeS_2) acting as light transducers. In this two-stages operation, the photosynthetic magnetic particles could be comparable to a modern photosynthetic system. A simpler particulate semiconductor system or "dual *n*-type model" has been proposed (Bard, 1980). The model is based on photoelectrochemical devices combining the photovoltaic with photochemical approach. Photovoltaic effects are produced in solar cells which convert sunlight into electricity. In *n*-type semiconductor materials conduction arises from the electrons introduced by cationic impurities. Oppositely in *p*-type semiconductors there is a deficiency of electrons but these "positive holes" can also act as charge carriers.

Another process of charge separation of physiological importance is that occurring across membranes of chloroplasts in light. This biomechanism of light-induced charge separation is akin to that intervening in man-made solar cells or silicon photovoltaic cells in which an electric potential develops across the p (positive) — n (negative) charges junction. Energization of the process of charge separation (positive "holes" versus electrons) is insured by the protons emitted under steady illumination of the crystalline silicon. Here also, the effectiveness of the photovoltaic cells rests on their inherent asymmetry and the ability of the junction (doped with trace elements "impurities") to keep the positive and negative charges separate. In the absence of photons, energy for charge separation in chloroplasts could be provided by a proton gradient artificially created by acidification (ΔpH) across their thylakoid membrane and leading to total dark synthesis of ATP (Jagendorf and Uribe, 1966). Resonance dipole-dipole interaction is the mechanism by which excitation energy is transferred in a pair of fluorophores with overlapping of emission/absorption spectra. Such nonradiative transfer of energy by a large number of donor labels to a small number of acceptors corresponds to an "antenna effect" (Morawetz, 1988).

In model membrane systems simulating photosynthesis, light-induced giant dipoles have recently been produced (Warman *et al.,* 1986). Here the charge separa-

tion was obtained within specially synthesized molecular assemblies in which a donor moiety was separated from an electron acceptor by a rigid, saturated hydrocarbon framework. Photon absorption by the donor moiety was followed by rapid electron transfer (Fig. 4 in II.C.2).

Polarization of opposite electric charges is basic in the conversion of solar energy into chemical energy. According to Calvin (1974), the primary quantum conversion apparatus of photosynthesis "in some way simulates a solid state device in which impinging light separates charges — positive and negative, electrons and holes". In that process whereby light energy from several photons is used to make a molecule of a reducing agent, many electrons and protons transfers intervene to prevent back-reactions by physically separating oxidized and reduced species.

Biological membranes should prevent back combinations of the oxidized and reduced species being produced. In the chloroplasts, this barrier role is ascribed to the highly structured and necessarily asymmetric thylakoid membranes. At the level of such engines of photosynthesis, a photon is caught in the first place by an array of a few hundred chlorophyll and carotenoid molecules which are located in the thylakoid membrane, where they act as antenna. The light energy is rapidly passed between the molecules by succesive re-emissions and absorptions until it arrives at a special chlorophyll molecule attached to a protein, where an excited electron and positive hole are created. These remain separated, the reducing effect of the electrons and the oxidizing effect of the holes being directed to opposite sides of the membranes. The barrier membrane is therefore a crucial component of energy transducers that depends on chemiosmotic effects; in these transducers, the picking up and dropping of protons concomitant with the changing back and forth between electron and hydrogen carriers, and the way in which these carriers are polarly arranged in the membrane, allows protons to be picked up mainly on one side of the thylakoid membrane and deposited on the other. Much of the energy of the excited electrons thus ends up as a proton potential difference across this membrane. ATP is then made in separate locations by allowing protons to flow back down this potential gradient. The thylakoid thus makes two high energy reagents — NADPH and ATP — which elsewhere in the chloroplasts are used in making carbohydrate from CO_2.

Further attempts should be made to more fully understand the molecular and subatomic principles by which chlorophyll and bacteriorhodopsin function as efficient traps for light energy, as well as how electric charges and H^+ can be separated across membrane to create an energy-rich electrochemical gradient. It is already known that, as the light-excited bacteriorhodopsin molecules in the membrane revert to their initial ground state, the energy released is harnessed to translocate H^+ ions from the inside to the outside of the cell, to form an acid-outside pH gradient across the cell membrane. In the reconstitution experiments first reported by Stoeckenius and Racker (Lehninger, 1982), inverted synthetic phospholipid vesicles have been prepared containing bacteriorhodopsin molecules

from *Halobacterium halobium* and the F_0F_1 ATPase from beef heart mitochondria. When illuminated, these vesicles externally synthesize ATP, again demonstrating (see IV.B.2c) the fundamental role of membrane polarity in bioelectrochemistry.

Electrogenic ion fluxes through photosynthetic membranes can be monitored via electronic band shift of synthetic pigments (Junge, 1977). Such shift, associated with light-driven photosynthetic reactions, results from the specific spatial arrangement of components involved in the electron transport within a functional membrane of chloroplast. The primary electron donors of photosystem II (P680) and photosystem I (P700) are located toward the inside of the thylakoid membrane, while the primary electron acceptor (pheophytin for photosystem II, chlorophyll for photosystem I) are located toward outside. Thus, when light causes the oxidation of the donor and reduction of the acceptor, a transmembrane electric field across the thylakoid membrane is established. As expected, chemical uncouplers alter the electrochromic band shift, both in chloroplasts and in chromatophore of photosynthetic bacteria. In photosynthetic or thylakoid membranes, photophosphorylation is driven by an electrochemical potential difference of the protons (Mitchell, 1966). The light-driven net uptake of protons into the thylakoid lumen is electrically balanced by the motion of other ions. A chloride channel has been detected which could provide a regulatory mechanism involved in charge-balancing during light-driven proton uptake by thylakoids (Schönknecht *et al.*, 1988).

The molecular machinery for catalyzing the primary storage processes of photosynthesis is contained within a protein complex embedded in a membrane and called a reaction center. The reaction center is excited by the energy of photon which is initially absorbed by an antenna system of many hundreds of pigment molecules. The energy of the absorbed photon is rapidly transferred by exciton migration to the reaction center which acts as trap.

A notorious achievement has been the cocrystallization of the membrane proteins and smaller molecules of the photosynthetic apparatus. To meet the challenge to crystallize such membrane proteins, detergents were first used to free the proteins from the membrane, but the key next step was to employ small "amphiphilic" molecules (hydrophilic-phobic elements) that effectively kick the detergents molecules away from the proteins and take the place of water in crystal lattice. This has permitted to apply X-ray crystallography to the analysis of the photosynthetic reaction center of the purple bacteria *(Rhodopseudomonadaceae)* and the determination of its structure. It is to date the only integral membrane protein whose structure is known to atomic resolution (Deisenhofer *et al.*, 1985; Youvan and Marrs, 1987). The three-dimensional structures of the photosynthetic reaction centres of Rhodobacteria have been determined, allowing a molecular description of the primary charge separation process (Fleming *et al.*, 1988). These studies indicate that there are two different symmetrically related possible pathways for the primary electron transfer to occur. As for the involvement of the protein in the electron

transfer process, it has been concluded that for the later step there is substantial coupling to low frequency polar modes of the protein (Bixon and Jortner, 1986).

The photosynthetic apparatus contains a large number of "antenna" molecules that absorb light and transfer the excitation energy by radiationless processes to the reaction centers. In cyanobacteria and red algae the antenna assembly is called the phycobilisome. This particle is a peripheral membrane complex made up entirely of proteins, most of which carry covalently bound, open chain tetrapyrrole prosthetic groups or bilines. Although phycobilisomes contain so many chromophores, the energy of a photon absorbed anywhere within the particle is transferred to the few terminal acceptors in an *uni*directional polarized-manner in a maximum of five to six kinetically significant steps (Glazer, 1985).

As modes of coupling between photoreceptors' relaxation processes and the macromolecular conformation, there are (Song, 1980): a) the polarizability, both isotropic and anisotropic, of the photoreceptor changes upon excitation by light; the utility of polarizability change, as a driving force for conformational change is probably very limited, although anisotropic polarizability may have a significant effect on the microenvironmental dynamics of the chromophore binding site; b) the change in excited state dipole moment of a photoreceptor upon light excitation which entails reorientation of the photoreceptor and/or surrounding residue dipoles of the protein or membrane.

E. Cytoskeletal components

The filamentous molecules of proteins that form the network-like structure of the hyaloplasm had been described some years before the use of the electron microscope as the "cytoskeleton" by Sir Rudolph Peters (Bourne, 1970). High voltage electron microscopy then permitted to reveal that the hyaloplasm is pervaded by a three-dimensional network of very fine threads, called the *microtrabecular lattice.* Its filaments of structural proteins radiate through the cytosol (see IV.A.2); they are interconnected and anchored to the plasma membrane and organelles. Collectively, with the cytoplasmic filaments and microtubules, the lattice forms the cytoplasmic (or cell) *matrix.* Less inclusive, the *cytoskeleton* comprises only the cytoplasmic filaments and the microtubules. The cytoplasmic filaments can themselves be divided into three classes (Sheeler and Bianchi, 1987): 1) microfilaments (thin filaments of 6 nm diameter) composed primarily of the globular protein G-actin polymerized into F-actin; 2) intermediate filaments (7-11 nm diameter) formed from 5 different classes of proteins, vimentin, desmin, cytokeratin, etc.; 3) myosin filaments (or thick filaments of up to 22 nm diameter) rich in the fibrous protein myosin. As for microtubules, they are much wider (about 25 nm), hollow structures made of α and β tubulins as major proteins.

While microtubules act essentially as radial guides for intracellular transport, microfilament rather dominate the outer part of the cytoplasm. They influence the characteristics of cell surface through a specialized actin-based scaffolding underlying the plasma membrane of certain cells. At the junctions of the microfilamentous network the short actin chains are interconnected by long molecules of a spacing factor, spectrin. The network is itself anchored by an other protein, ankyrin, which binds the spectrin cross-links to a major transmembrane protein (Weber and Osborn, 1985).

Immunofluorescence microscopy has contributed to unravel the configurations of these cytoskeletal filament systems: microtubules radiating from the cytocenter (around the nucleus) may act as radial guides for intracellular transport in the non-dividing cells; for cell division they break down and the tubulin is reassembled into the microtubules of the mitotic spindle (see IV.F.2).

Filamentous polymers of the acidic, Ca^{2+}-regulated, contractile proteins (actin, myosin) and tubulin have a polarity due to the arrangement of their asymmetric subunits in a specific orientation in the polymer. The structural polarity of the polymer is essential in both muscle and cilia for the production of organized movement. Microtubules also play a role in establishing the leading edge or lamellum of motile cells and maintaining cell polarity (Alberts *et al.*, 1983). The cytoskeletal framework is therefore made of filamentous structures which are polar as their heads and tails are chemically distinct. By providing such an anisotropic and asymmetric, three-dimensional setting for the molecular vital events, it may influence the polar movement of organelles (Weber and Osborn, 1985) and be considered as responsible for intracellular spatial organization (Fulton, 1984). However, remains the fundamental question (Mitchison and Kirschner, 1984) peculiar to the macroscopic organization of the cytoskeleton "By what means does the cell localize polymerization in certain positions and along certain directions and at the same time prevent random polymerization of cytoskeletal elements?"

1. Microfilaments-actin

It is well recognized that actin filaments are structurally polar. This polarity can be readily determined by "decorating" actin filaments with myosin subfragments (S1 and heavy meromyosin). Each actin molecule in an actin filament is capable of binding one molecule of S1. It is the complex of actin filaments with S1 that reveals the polarity of the actin filament. In negatively stained preparations in the electron microscope, such complexes have a regular and distinctive form: each S1 fragment forms a lateral projection, and the superimposed image of many such projections gives the appearance of arrow-heads along the actin filament. The arrows on these *decorated actin filaments* all point the same way on both strands of the filamentous

actin double helix, demonstrating its inherent structural polarity. Elongating actin filaments are thus *bi*polar and have different, "barbed" and "pointed" ends as determined by the appearance of the heavy meromyosin labelled filaments (Huxley, 1963). Actin monomers add more rapidly to the barbed filament end (+) than to the pointed end (−). This difference in the critical concentration dictated by the opposite ends of actin filaments is consistent with the idea that actin monomers might cycle through the filaments from the (+) end to the (−) end by the process called "head-to-tail polymerization" or "treadmilling" (Wegner, 1976; Neuhaus *et al.*, 1983; Fig. 13 A).

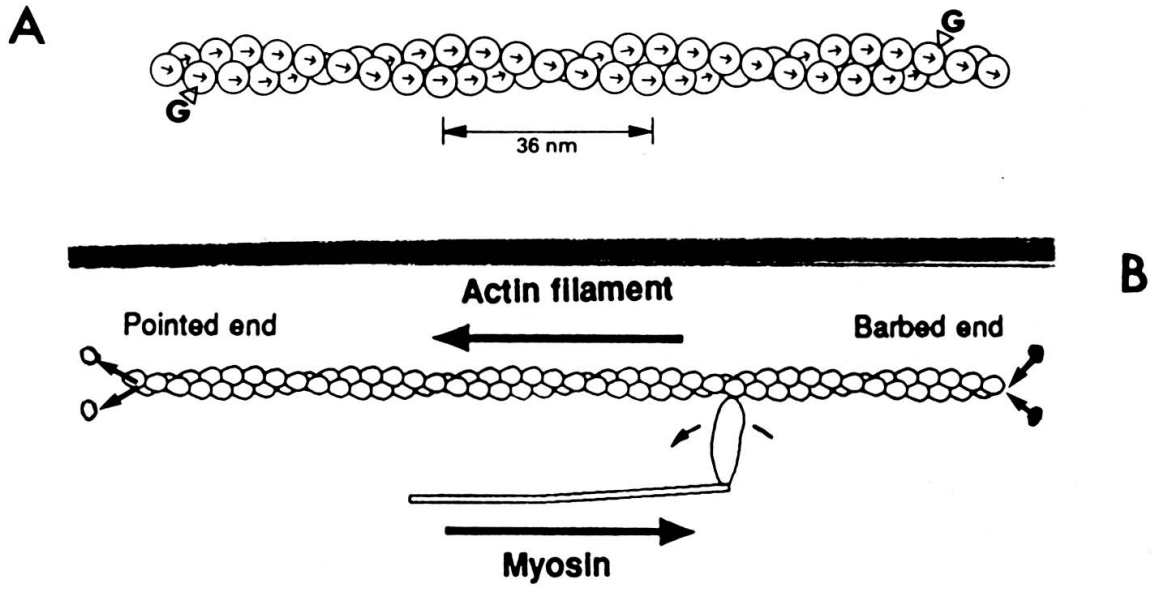


FIG. 13.

Structural and functional bipolarity of actin.

(A) Polarized polymerization of the globular (G) protein monomers into a F-actin microfilament. Adapted from Darnell *et al.* (1986).

(B) Treadmilling process of an actin filament polymerized from its barbed (+) end, with example of inversely displacing myosin (arrow) through a hook. Adapted from Smith, Science, 242: 708 (1988).

Another way to determine polarity of microfilaments is to inhibit the growth at one end of the polymer with a small ligand of protein and then measure polymerization rate from the other end. An end can be capped by a protein such as brevin to form the actin-brevin complex serving as a nucleus that growths only from one end (Frieden, 1985). Cytochalasins have been found to prevent growth from the *fast*-growing end (Tannenbaum, 1978). Now, it would be interesting to undertake capping of the *slow*-growing end of the actin filament. However, and contrarily to microtubules (IV.E.3), these actin filaments are not organized in a manner that provides satisfactory cues for directional movements between the center and periphery of the cell (Sanger and Sanger, 1980).

Multistep polymerization of ATP-actin occurs through a nucleation (primer nucleus) elongation process. Polymeric F-actin is formed by reversible non-covalent self-association of monomeric G-actin. During the polymerization process ATP is bound to G-actin and then hydrolyzed to ADP that binds to F-actin. There are potential mechanisms for regulating this monomer-polymer transition: at high rates of filament growth a transient cap of ATP-actin subunits exists at the ends of elongating filaments, and at steady state a stabilizing cap of ADP.P_i-actin subunits exists at the barbed ends of filaments (Korn *et al.*, 1987).

Passed the nucleation process, the polymerization rate of actin (or tubulin, see E.3) should reflect the elongation process. Attempts have been made to measure this elongation rate for actin under selected conditions and using calibration of the system with electron microscopy (Pollard, 1983). From further experiments, Pollard (1984) has proposed that Ca^{2+} which binds to low affinity sites influences this polar process.

Polymers of actin and tubulin have a polarity due to the arrangement of their asymmetric subunits in a specific orientation in the polymer. The structural polarity of the polymer is essential in both muscle and cilia for the production of organized movement. It also makes the two ends of the polymer different in a way that is important for the control of polymer growth. The polarity of F-actin is revealed by its polymerization, at one end, the plus end, and its depolymerization, at the other end, the minus end; the flow of protein subunits of G-actin involved in such a treadmilling is regulated by capping molecules which are specific for either the plus or the minus end of the filament. Then, by controlling the local shortening and lengthening of F-actin filaments and therefore its state of polar polymerization, the capping proteins play a part in intracellular movements.

Actin molecules self-assemble to form a large variety of three-dimensional structures that influence cell form and function. The regulation of this assembly of actin is ascribed to proteins called "actin-binding proteins". The most dynamic regulatory actin-binding protein is myosin which binds to filaments of opposite polarity to produce contraction and thus acts as the motor of cell movements (Fig. 13 B). Among the other binding molecules known (Stossel *et al.*, 1985) there are those which act by predominantly: a) sequestering actin molecules (profilin, etc.); b) blocking the ends of actin filaments (gelsolin, villin, severin, etc.); c) cross-linking actin filaments with promotion of isotropic networks (filamin, spectrin, etc.), bundling effect (α-actinin, fascin, fimbrin, etc.) or gelling of actin (gelactins, etc.) which implicates a thixotropic equilibrium G-sol – F-gel; d) sides-binding actin filaments (tropomyosin, cofilin, etc.).

In order to exert a mechanical force, a contractile assembly must be anchored to other cellular components. The question then arises, what is the polarity of the actin filaments whose tips are "attached" to membranes? This question is important in two respects (Stossel *et al.*, 1985). First, if the polarity is unidirectional with respect

to the membrane, one must postulate that there are components at the tip of the actin filaments which not only glue the filaments to the membrane, but which also regulate the polarity of the attached actin filaments. Second, if cytoplasmic myosin is to interact with these membrane-attached filaments, then by knowing the filament polarity we immediately know whether or not the membrane can be pulled toward the cell center by myosin. In fact, most actin filaments are anchored at one end to cell membranes. This anchorage is also true for noncontractile actin assemblies: for example, the actin filaments in the core of a microvillus are attached to the overlying plasma membrane both at their tips and along their lengths. Many of the actin filaments are nucleated with the same intrinsic polarity from sites on the plasma membrane. The polarity of the actin filaments with respect to the membrane has now been determined in a variety of cell types. In all cases the arrowheads point away from the plasma membrane toward the cell center. At odds with this directionality is a report which shows that, in motile blebs of corneal epithelial cells in culture, the polarity of the actin filament attached to the membrane is opposite: the arrowheads point toward the membrane (Sugrue and Hay, 1981). Why the actin filament in these blebs have a different polarity from those in all the other systems remains obscur.

Arrays of "stress fibers" or long, thick bundles of microfilaments running parallel to and just inside the plasma membrane have also been revealed by fluorescent antibodies for actin. In cultured cells, these stress fibers terminate at specialized regions of the plasma membrane, known as *adhesion plaques*. Interestingly, rounded tumoral cells lack such stress fibers (Weber and Osborn, 1985).

2. Microfilaments-myosin

Myosin can be extracted from skeletal muscle by treatment with concentrated salt solutions and each molecule can be broken down by treatment with high concentration of urea or detergent into six polypeptide chains: two identical *heavy* chains and two pairs of *light* chains. Purified myosins consist of two globular heads joined by an extended α-helical coiled-coil tail. Each head (S1 subfragment) has an actin binding site and exhibits actin-activated ATPase activity which is essential for movement. Schematically, conventional myosin is thus a hexameric protein which comprises the two heavy chains of its tail and the four light chains of its head (Warrick and Spudich, 1987).

The body of the natural thick filament is composed of numerous myosin tails from which the myosin heads project in a repeating pattern. That structure is bipolar (Fig. 14 A), with the bare central region where two oppositely oriented sets of myosin tails come together. The globular heads of the myosin molecule interact with actin and form the cross-bridges between the thick and thin filaments of muscle.

Myosin is an actin-activated ATPase and it is by its head that it binds to actin filaments. As such it is the main actin-binding protein. It converts the chemical energy

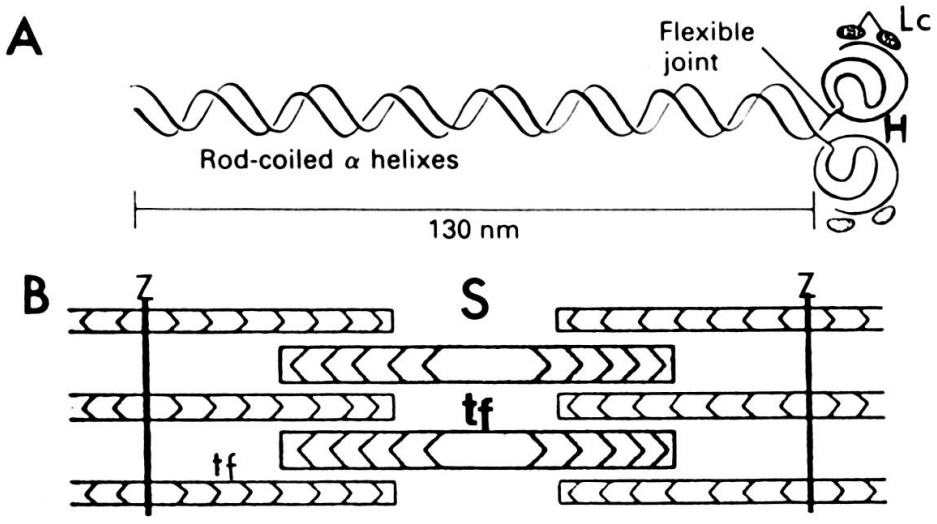


FIG. 14.

Myosin polarity. (A) Bipolarity of a thick filament. H = myosin head; Lc = light chain. Adapted from Darnell (1986). (B) Schematic diagram of a muscle sarcomere (S) showing that the thick (**tf**) and thin filament (tf) overlap with the same relative polarity on either side of the midline. (Z = midline). Note reverse polarity at midline of thick filaments. Adapted from Alberts *et al.*, 1983.

of ATP into contractile movement. Myosin molecules stretch and aggregate into small bipolar filaments under the coordinate action of myosin kinase, a microfilament-associated protein, and calmodulin, a calcium-regulatory protein. In the process of contraction the bipolar myosin filaments bind at both ends to properly aligned actin filaments having identical polarity; in the presence of ATP they then slide filaments along each other. Direct proof of this ATP conversion into mechanical work was experimentally provided by quantitative measurement of rates of movement of purified myosin along actin in vitro first made by using the *Nitella* based movement assay of Sheetz and Spudich (1983). In that assay, myosin filaments are attached to polystyrene beads. The myosin filaments being bipolar are bound to the beads in random orientation and the beads move in only a single direction — that is defined by the polarity of the actin filaments. Single actin filaments stabilized with fluorescent phalloidin have been shown to exhibit ATP-dependent movements on myosin filaments from both skeletal muscle and slime mold *(Dictyostelium)* fixed to a glass surface (Kron and Spudich, 1986). Using this myosin-coated surface assay, Harada *et al.* (1987) showed that single-headed myosin filaments bound to glass support movement of actin at nearly the same speed as intact myosin filaments. According to this study the terminal portion of the rod and the two-headed nature of myosin are not required for movement. Interestingly, a single-headed myosin is a normal constituent of the protozoan *Acanthamoeba.* Its short tail is distinct from that of usual myosin, but it supports movements in the *Nitella* assay (Albanesi *et al.*, 1985).

Folding of the myosin molecule is concomitant with its ATP hydrolytic activities. Transition forms to the folded state have provided clues about filament construction. A stable 15S folded dimer apparently unfolds to form an antiparallel dipolar unit with a tail overlap of 40-50 nm (Trybus and Lowey, 1987). Such a building unit would explain the characteristic absence of a central bare zone and the "mixed" or "site-polar" appearance of long filaments assembled from smooth and nonmuscle myosins (Craig and Megermann, 1977).

There is one aspect of polymorphism in myosin filaments that is of distinctive significance: at the center of the thick filament, molecules which point in opposite directions are bonded together; growth proceeds at either end by association of molecules pointing in the same direction (Huxley, 1963). Thus the specific contacts in the initiating dimers are not the same as those in the polar portions, since the molecule itself is polar. This dimorphism determines the *bi*polarity of the thick filament which is fundamental to the sliding filament contractile mechanism. The same kind of dimorphism is observed with paramyosin which forms the core of the very thick filament in molluscan "catch" muscle. Paramyosin is an α-helical protein similar to myosin, but lacking the globular portions. This protein can also assemble itself into bipolar filaments in which the molecules have an antiparallel arrangement at the center and a parallel packing at the polar ends (Kendrick-Jones *et al.*, 1976). The type of "positional" polymorphism displayed by myosin and paramyosin differs from that of icosahedral virus coat proteins which conserve the local bonding relations.

In muscle cells myosin forms the typical, thick bipolar (double-headed) filaments associated with F-actin to form orderly contractile units, the actomyosinic sarcomeres. The myosin head regions (the S1 fragment) have opposite orientations on either side of the bare region of the thick filaments that is, on either side of the sarcomere A band (Fig. 14 B). Since these head regions must interact with the thin filaments in the region of overlap, it would be reasonable to assume that the thin filaments on either side of the sarcomere are of opposite polarity. According to Huxley's swinging cross-bridge model (1969) for the molecular basis of muscle contraction, the myosin head undergoes a change in conformation while bound to the actin filament, resulting in a step of relative displacement of the actin and myosin filaments (Fig. 13 B; also in Warrick and Spudich, 1987).

In nonmuscle cells myosin is in a far less ordered state only detectable among the microfilaments by electron or immunofluorescence microscopy (Weber and Osborn, 1985). The relatively small size of the filaments formed by nonmuscle myosin is only one of three important characteristics that distinguish it from skeletal muscle myosin. The others are that the activation of nonmuscle myosin, as with smooth muscle myosin, depends on the phosphorylation of the myosin light chains and that phosphorylation induces the assembly of nonmuscle molecules into small bipolar aggregates in which 10 to 20 myosin molecules (compared with 500 or so myosin molecules in a skeletal muscle thick filament) are held together by the aggregation

of their tail regions. As with smooth muscle myosin, phosphorylation is catalyzed by a myosin kinase that is stimulated by Ca^{2+}, so that both the state of aggregation of nonmuscle myosin and its ability to interact with actin filaments are altered by small changes in the concentration of free Ca^{2+} in the cytosol. Such changes in intracellular Ca^{2+} commonly occur in response to extracellular signals.

How can actin and myosin produce coherent movements when the filaments are distributed in an apparently random three-dimensional network? At least at one level, the answer seems to be that an actin filament has a well defined polarity and that myosin heads can bind and move along an actin filament only if they are oriented in the correct direction with regard to the filament's polarity. Thus the small bipolar aggregates of nonmuscle myosin molecules also probably form miniature sarcomeres by pulling one set of actin filaments against another, even though the actin filaments and myosin aggregates are not part of a highly ordered array.

The position of the ATPase site of myosin has been determined using three-dimensional image reconstruction from electron micrographs and site-specific labelling with the avidin-biotin system. The ATPase site is about 5 nm from the tip of the myosin head and is about 4 nm away from the actin-binding site of myosin (Tokunaga *et al.*, 1987). This is the first report of the three-dimensional location of an enzyme active site by electron microscopy.

The locomotory role of myosin has been examined in the cellular slime mold *Dictyostelium* using gene targeting to interrupt the single copy *mah*cA myosin gene. Upon integration of a plasmid, a cell was created in which the intact myosin molecule was replaced with an amino-terminal fragment incapable of forming filaments. The extremely large mutant cells still grew with movements resulting in pieces of the cell being pinched off (De Lozanne, 1987; De Lozanne and Spudich, 1987). Recently, we have obtained electrophoretic evidence, in cell-free extracts of *Neurospora*, for the presence of a myosin-like protein (Hoang-Van and Turian, 1987), thus conforting our scheme for polarized hyphal growth (Fig. 21).

3. Microtubules-tubulins

Microtubules are long, thin, hollow cylinders made of the protein tubulin and various microtubule-associated proteins (MAPs), including tau protein. Along with other fibrous components, they provide the cell with an ever changing three-dimensional framework. They play various structural and motor roles in every eukaryotic (or nucleated) cell. As such they are the main structural members of motor organelles as cilia and flagella. They also function, either as cables or draglines or simply as tracks, to move things from place to place in the cell. A mechanism that has lately come to be understood may explain how the microtubules accomplish some of these tasks. In certain contexts, at least, it seems that a microtubule can act as a treadmill or an escalator and might thereby carry attached materials along in its motion.

Microtubules are polymers of the protein tubulin, which itself is a double molecule composed of two similar but distinct globular polypeptides, alpha and beta tubulins. The assembly of tubulin (GTP-directed in vitro polymerization) takes place as dimers of α- and β-tubulin linked to form 13 protofilaments (strands) arrayed around a hollow core. The resulting microtubule (22 nm diameter), when unrolled, shows a pattern of bipolarity arranged dimers (Fig. in Weber and Osborn, 1985). Most microtubules are extremely labile: they are continually being assembled from a cellular pool of tubulin subunits and then disassembled.

The process of assembly and disassembly has been studied primarily in laboratory preparations rather than in living cells. It became clear that subunits are added to and removed from the microtubule at both ends. A few years ago Gary Borisy and his colleagues (1974) found that when the microtubule is growing, the rate of growth is different at the two ends: there is an intrinsic polarity of growth. In 1978, Robert Margolis and Leslie Wilson discovered a related phenomenon under steady-state conditions, that is, when the amount of tubulin incorporated into microtubules and the amount of free in the medium are in equilibrium. Under these circumstances the overall rates of assembly and disassembly must be equal, but Margolis and Wilson found that subunits are mainly added at one end, and mainly lost on the other end (Fig. 15 A). The result is a unidirectional flux of subunits along the microtubule.

Microtubules are intrinsically polar fibres (Allen and Borisy, 1974; Margolis and Wilson, 1978). Unlike microfilaments in which directionality can be visualized directly by specific interaction with heavy meromysin, the polarity of microtubules has, until now, been approached only by more devious methods. The most successful has exploited the ability of tubulin subunits with attenuated capacity to self-assemble, to polymerize *in vitro* onto some preformed seed or organizing center. Various cellular structures will apparently serve although most frequently used are flagellar axonemes from the green alga, *Chlamydomonas,* whose two ends are morphologically distinguishable. This directional polymerization is taken to reflect an intrinsic molecular polarity within the microtubule structure, that is, the asymmetric tubulin subunits are all oriented in the same way along the microtubule axis, an arrangement also inferred from detailed analyses of microtubule structure by electron microscopy and optical diffraction (Amos and Klug, 1974).

The tubulin dimer has two nucleotide binding sites, one of which is nonexchangeable and invariably contains GTP in some structural role (Weisenberg *et al.,* 1968). The exchangeable site can bind both GTP and GDP, but the dimer will only polymerize readily into microtubules when liganded with GTP (Weisenberg, 1972). When the tubulin dimer polymerizes, the GTP is hydrolyzed to GDP with release of free energy. On the basis of an analysis of the exchange of [^{3}H]GTP with microtubules assembled to steady state, Margolis and Wilson (1978) have proposed a novel mechanism of microtubule assembly whereby subunits are added to one end

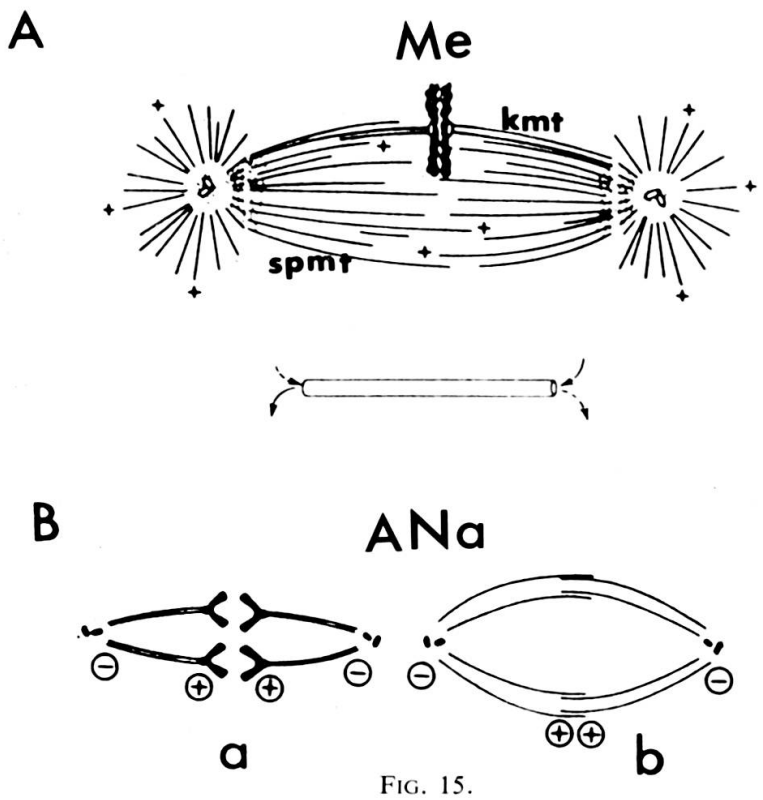


FIG. 15.

Polarity and the microtubule-chromosome complexes.

(A) Sketch of the current concept of microtubule arrangements in a metaphase (Me) first-division sea urchin spindle, with only one chromosome pair drawn for clarity. All the microtubules in each halfspindle of the same polarity: minus ends within the polar centrosomal material, plus ends attached to the kinetochore or lying toward the mid-plane. Below, treadmilling or head-to-tail polymerization of a spmt; spmt = spindle pole microtubules; kmt = kinetochore microtubule. Adapted from Salmon (1984), Cold Spring Harbor Sympos., U.S.A.

(B) Anaphasic (ANa) separation of the mitotic spindle poles based on the intrinsic polarity of the microtubules: (a) kinetochore microtubules shortening to move the chromosome toward the pole; (b) pole-to-pole microtubules with antiparallel sliding. Adapted from Koshland *et al.*, 1988.

of the microtubule and removed at the other in a unidirectional (opposite end) assembly and disassembly process. The result is a continuous flow, or "treadmilling" of subunits along the microtubule at a rate measured to be 0.69 μm h^{-1}. The process of treadmilling of tubulin molecules in a microtubule implies that, at equilibrium, tubulin molecules (as αβ heterodimers) are continually adding to and being lost from both ends of the microtubule. In the presence of GTP, however, there is a net addition at one end (+) and a net loss from the other end (−). Consequently, there is a continual movement of tubulin from one end of the microtubule to the other. A similar situation may exist for actin filaments (Fig. 13) in which treadmilling is driven by ATP hydrolysis.

The polymerization reaction of microtubules can be broken down into a series of rate constants for the partial reactions, and depending on the relative rates, different dynamic behavior is possible, of which one particular case would be "treadmilling" (Hill and Kirschner, 1982). As for the depolymerization reaction, it is not

simply the reverse of the polymerization and, in particular, depolymerization is much faster than expected by extrapolation of the polymerization rate at different tubulin concentrations. Carlier *et al.* (1984) hypothesized that growing microtubules have GTP subunits at their ends that have not yet hydrolyzed, forming a GTP-liganded cap. GTP cap is therefore necessary to stabilize a microtubule end and its loss leads to the initiation of depolymerization. The presence of GTP cap can mean that a microtubule array is composed of two phases, growing and shrinking microtubules. Since these phases seem to convert infrequently, the population is extremely dynamic. In particular, at steady state, the population of microtubules consists of a majority growing slowly and a minority shrinking quickly (Mitchison and Kirschner, 1984). If this type of unusual dynamics, termed dynamic instability, also occurs in cells, it provides a special role for microtubule-organizing centers (MTOCs). This role is essentially concerned with the hydrolysis-induced polymer destabilization as known from the early work with lysed cells and mitotic lysates (Brinkley, 1985). Recently, several lines of evidence have also suggested an interaction between MTOC and the Golgi apparatus (Kreis *et al.*, 1988).

The microtubules that emerge from MTOCs have an intrinsic molecular polarity based on the sequence of α- and β-tubulin subunits along the protofilaments. They also have the + and − polarity (see above), which may or may not be related to this intrinsic molecular polarity, or to the conformation with respect to a MTOC, or to the direction in which mechanochemical forces might be generated along the microtubules (Gunning, 1981).

Many drugs among which colcemide, benomyl, nocodazole can disturb assembly and normal arrangement of microtubules. Oppositely, taxol promotes rather than inhibits the polymerization of tubulins. Such drugs have been profitably used to provide evidence that microtubules define spatial order within the cell. Intriguing as these assembly studies might be, they are not the sole experimental handle on microtubule directionality. The long-awaited morphological marker of microtubule polarity was then unraveled as dynein (Haimo *et al.*, 1979), the ATPase protein responsible for the movement of cilia and flagella, and seen as paired projections or arms connecting the 9 + 2 microtubules. Haimo and her colleagues showed that axonemal dynein from *Chlamydomonas* added to microtubules during or subsequent to assembly binds periodically along the length of the microtubule, forming a complex reminiscent of the "decoration" of microfilaments by heavy meromyosin.

When techniques for in vitro microtubule assembly were developed, they were used to determine that the two ends of flagellar microtubules demonstrate different assembly polarity, i.e. the proximal and distal ends of axonemes nucleate microtubule assembly in vitro at different rates (Allen and Borisy, 1974; Binder *et al.*, 1975). For both outer doublet and central pair microtubules, assembly of chick brain tubulin occurred five times faster at the distal end than at the proximal end. This polarity was confirmed by Euteneuer and McIntosh (1981*a*) using a method in which partial

microtubule sheets are assembled onto preformed microtubules to determine the intrinsic polarity. The partial microtubules form "hooks" whose orientation indicates the polarity of the template microtubule. Using this method it was shown that all of the A and B subfibers of the outer doublets of an axoneme, as well as the central pair microtubules, are oriented in the same direction, with the *plus* end (that with the highest assembly rate) distal to the basal body. Thus, if central pair microtubules grow from their proximal end, this growth occurs at the slow growth end.

Microtubules take part in several mechanisms of intracellular motility including organelle transport and mitosis (see IV.F). The late Robert Allen was the first to see individual microtubules sliding and squiring over a glass surface, using a high-performance video camera attached to light microscope. These studies arose from his early interest in the movement of particles in the filopodia of a marine foraminifer. Using such advanced techniques, Allen (1985) observed movement of numerous small organelles (less than 200 nm) in the *anterograde* (towards the cell terminal) and *retrograde* (towards the cell body) directions in the intact squid axon. As nicely illustrated by Allen (1987) «microtubules mediate the transport of vesicles and organelles through the corridor that connects the cell body to the terminal extension of the cell». This is what occurs in the axonal transport along microtubules allowing the swift exchange of substances between the nerve cell body and the synaptic terminal at which the nerve fiber adjoins its target cell (see IV.B.2f). Movement of mitochondria which occurs in both antero- and retrograde directions is intermittent contrarily to the continuous transport of the vesicles. A single microtubule can support transport in both directions. In his attempts to understand the transport mechanism Allen (1987) detected an interactive protein on the surface of the microtubule; this protein bears sidearm or cross bridges transiently connecting vesicles and organelles. For his active-native-microtubule model, Allen (1987) proposed that each sidearm contains a force generating ATPase producing conformational change from an «activated» state to a «resting» state and forming transient cross bridges between the vesicle and the microtubule. The ATP-generating force is thus utilized to move the vesicle along the microtubule — or to move the microtubule along an adjacent surface. Alternate «backstroke» hypothesis and «walking» mechanism have been proposed and illustrated by Allen (1987) to explain such organelle movement in constant association with the microtubules.

The uniform orientation of microtubules indicates that microtubule polarity could serve as a «compass» by which particles could navigate in the cytoplasm» (Vale, 1987). Thus an organelle could only be directed toward the interior (minus end) or periphery (plus end) of the cell by the polarity of the microtubule lattice. Therefore, for antero-versus retrograde organelle transport there was a need for polarity-specific translocators as determinants of this organelle traffic direction. By the use of isolated components of such motility systems, it has been possible to isolate and biochemically

characterize the mechanofactors at work in these motility processes as «motor proteins», the best known being kinesin (Vale *et al.*, 1985*a*, 1986; Hollenbeck, 1988).

However, is kinesin the only microtubule-based, force-generating protein motor for organelle transport in cells and axons? According to Vale *et al.* (1985*a*) the answer to this question is «apparently no, since kinesin is only able to induce movement towards the (+) ends of microtubules». The concerted action of kinesin with a retrograde translocator had to be envisaged to promote the *bi*directional movement of vesicles along microtubules. A second, distinct protein in axoplasm was described that promotes the translocation of beads in the opposite direction than that promoted by kinesin (Vale *et al.*, 1985*b*). This retrograde motor has recently been isolated as the cytoplasmic dynein (Vallee *et al.*, 1988), a protein distinct from the dynein responsible for the beating of cilia and implicated in mitosis. Both kinesin and dynein were identified as force-generating enzymes on the basis of their ability to promote the movement of glass or plastic beads on microtubules. However, the role they play in vivo is now being actively investigated in attempts to describe other «actors which may act by forming an organelle motor complex» (Schroer *et al.*, 1988).

Are there other microtubule motors? This question has been addressed by Vallee and co-workers at the last International Congress of Cell Biology (Montreal, 1988). A newly isolated protein also showed ATP-sensitive binding to microtubules (possible ATPase activity but relative insensitivity to vanadate). Thus these authors might have found another mechanochemical enzyme driving the microtubule-coupled cell movement.

F. Nuclei and mitotic figures

1. Interphasic and mitotic structures

Resting eukaryotic nuclei are surrounded by a double membrane which is interrupted by nuclear pores which are formed by the fusion of two membranes. The trilaminar structure from membrane around the edges of the nuclear pore suggests a direct continuity of the lipid bilayers of inner and outer membranes in this region. The rim of the pore contains eight granular subunits symmetrically arranged on the outer cytoplasmic margin of the pore (see Fig. in Sheeler and Bianchi, 1987). The pore complex contains three prominent substructures: rings (annuli), central spokes, and central granules (plug). The rings, spokes, and their connecting elements together form an assembly with two axes of symmetry. However, "it is unlikely that the nucleoplasmic and cytoplasmic rings are structurally identical since the pore complex probably is functionally asymmetric" (Gerace and Burke, 1988).

Electric, and therefore polarization phenomena, might participate in the exchanges processes through nuclear pores (Bourne, 1970). Recent work suggests that

the pore complex translocates proteins and RNAs between the nucleus and the cytoplasm by highly selective vectorial processes (Gerace and Burke, 1988). Experimentally, it has been shown that RNA isolated from the cytoplasm of eukaryotes, after injection into and implantation of a nucleus, is transported from the nucleus to the cytoplasm a second time. This indicates that the specificity of mRNA transport is not determined by precursor molecules, but rather should be looked for in the polarity of the nuclear pore complexes.

Differential light polarization microscopy by linear and circular differential imaging have been used by Mickols *et al.* (1987) to study the structures of the nucleoli in live primary spermatocytes of *Drosophila* when they are transcriptionally active or inactive. Some inactive nucleoli are bipartite, with two distinct structures visible by differential scattering of both linearly and circularly polarized light. According to the images obtained, the presumed granular region (a region of maturation of ribosomes) is arranged in a specific chiral arrangement not seen before.

The interphase centrosome is a cytoplasmic microtubule-organizing center which initiates microtubules with a well-defined polarity from a specific cellular position. It usually lies besides the nucleus, often displacing the nucleus from the center of the cell. Many centrosomes contain centrioles, but such an association is not universal. The centrioles generally do not attach directly to the cytoplasmic tubules of the cytoskeleton. In higher plant cells there is no obvious centrosome or centrosome analogue, during either interphase or mitosis. The mitotic centrosome function is present but the only structural correlate to the microtubule-organizing activity of the spindle pole is an accumulation of membranous vesicles (Jackson and Doyle, 1982).

The mitotic spindle is the fibrillar apparatus that moves the two sets of chromosomes toward opposite poles of a dividing cell. It is composed of a bipolar arrangement of microtubules. Mitotic microtubules that extend away from the region of the centrosome and spindle poles in vivo all have "plus" distal structural polarity, similar to the structural polarity of microtubules that have been nucleated from centrosomes in vitro (Telzer and Haimo, 1981; McIntosh and Euteneuer, 1984).

The mitotic spindle is therefore composed of two kinds of microtubules: a set of interpolar microtubules which extends from each pole toward the other pole, overlapping at the cell's equator; a set of kinetochore-to-pole microtubules which extends from each pole to the kinetochore, or point of attachment of each chromosome (Fig. 15). In each half-spindle both kinds of microtubules have the same polarity; the model proposed by Margolis and Wilson (1978) assumes that disassembly ends are at the poles.

Mitosis should be best studied with cells that are accessible to different methodological approaches. Immunofluorescence studies provided insight into the overall formation of the microtubular spindle especially in live myxamoeba of *Dictyostelium discoideum* (Roos, 1987). Electron microscopy revealed in the same

"naked" cells that as a spindle elongates, the number of microtubules decreases while the remaining microtubules elongate by addition of subunits at their distal, overlapping ends. Roos considered that his results "are compatible with the idea that the spindle elongates by microtubule sliding and elongation".

2. Polewards chromosome movement

During nuclear division or mitosis, polar processes intervene toward the end of prophase (prometaphase) when the spindle extends between two poles positioned diametrically opposite one another in the cell and the chromosomes migrate toward the center of the spindle. Then, at the onset of anaphase, the chromosomes move toward opposite poles of the spindle. As the centromere of each chromosome advances polewards, with the chromosomal arms lagging behind, this arrangement has suggested that the chromosomes are being pulled toward the spindle poles. Three major mechanistic models have been proposed to explain the polewards movement of chromosomes. Summarized from Sheeler and Bianchi (1987, pp. 501-504), they are: 1) The microtubule model (McIntosh, 1984) implicating extension of the microtubules inward from the spindle poles and their overlapping at the center of the cell; the poles are pushed further apart when the overlapping ends of the microtubules slide past each other as a result of disassembly of other microtubules connecting the centromeres to the poles, the overall length of these microtubules is decreased and the chromosomes move closer to the poles. 2) The sliding cytoplasmic filament model (Forer, 1974) accounting for chromosome movement by the sliding of actin and myosin filaments past one another. Microtubules only act as a structural framework on which the chromosomes are mounted. At onset of anaphase, their disassembly at the two poles of the spindle frees the chromosomes to move in response to the sliding of the actin and myosin. 3) The model of dynamic equilibrium (Inoué, 1967) between the spindle microtubules and a pool of microtubule subunits. The addition of new subunits to chromosome-free microtubules extending from one pole to the other serves to increase the interpolar distance. Parallely, the removal of subunits from either the polar ends or the centromere ends of other spindle fibers serves to shorten them and slowly draw the chromosomes closer to the poles.

Two kinds of motion are thus observed in mitosis: the poles separate and the kinetochore microtubules shorten (Fig. 15 B). In one of the more recent models proposed, Margolis and Wilson (1978) suggested that opposing sets of treadmilling interpolar microtubules slide or ratchet past each other, separating the poles. At the same time the chromosomes are reeled in toward the poles as the kinetochore microtubules (whose assembly at the chromosome end has been blocked) disassemble at the polar end. A critical factor in understanding the mechanisms that move chromosomes and regulate the assembly of mitotic spindles is identifying the pathways by which tubulin subunits exchange with microtubules in mitotic spindle fibers. Four possible pathways for the exchange of tubulin with spindle microtubules

in vivo have been proposed. All but one of the proposed pathways (Inoué and Sato, 1967; Bajer and Molé-Bajer, 1975; Inoué and Ritter, 1975) are based on end-dependent subunit exchange models, the mechanism by which microtubules apparently assemble in vitro (Hill and Kirschner, 1982; Mitchison and Kirschner, 1984).

Recent observations concerning microtubule polarity in cytoplasmic structures suggest an important role for it in determining the polarity of kinetochore movements but this could not be established. Another possibility is that movement polarity is built into the chromosome so that sister kinetochores point in opposite directions. If the motors are associated with the kinetochore or with the fibrous coronae surrounding the kinetochore, the inherent polarity of the chromosomes would favor an opposite pole attachment for sister kinetochores. Syntelic orientation of sister kinetochores (sister kinetochores attached to the same pole) would be disfavored because of the stress that this orientation would place on the chromosome.

There are frequent instances in cellular morphogenesis when a cell has to direct microtubules toward a particular part of the cell, e.g., into an extending neurite or into a pseudopod during locomotion. The structure of the centrosome does not suggest any mechanism by which it would direct nucleated microtubules. Instead, Mitchison and Kirschner (1984) suggest that given the dynamic nature of microtubules, the centrosome may continuously nucleate microtubules in random directions. If proteins that could cap or stabilize the ends of microtubules become localized in a particular region of the cell, the microtubules growing in that direction would become preferentially stabilized. Microtubules in other regions would continue to initiate depolymerization stochastically. Eventually, the majority of the cell's microtubules would be directed toward the stabilizing region and the cell would become polarized (Fig. 16).

One of the most important questions about spindle structure concerns the origin of the polarity of spindle microtubules. Whether chromosome movement is driven by proteins associated with microtubules, e.g. dynein-like molecules, or by dynamics of the microtubules themselves, the direction of this movement is probably specified by the polarity of the microtubule lattice. The properties of microtubules, centrosomes, and kinetochores can be combined to give a model that accounts for the uniform polarity found in the astral microtubules and kinetochore fibers at metaphase (Heidemann and McIntosh, 1980; Euteneuer and McIntosh, 1981*b*).

Dynein has been implicated in mitosis because known inhibitors of axonemal dynein ATPase (vanadate, etc.) inhibited anaphase motion in lysed cells. There is also some biochemical evidence for a molecule like axonemal dynein heavy chain present in sea urchin embryos during mitosis (Vale, 1987).

There are several features of microtubule polymerization that may be related to the need for specific localization of assembly. Microtubules in cells polymerize from organizing centers, the most prominent of which is the centrosome, a region

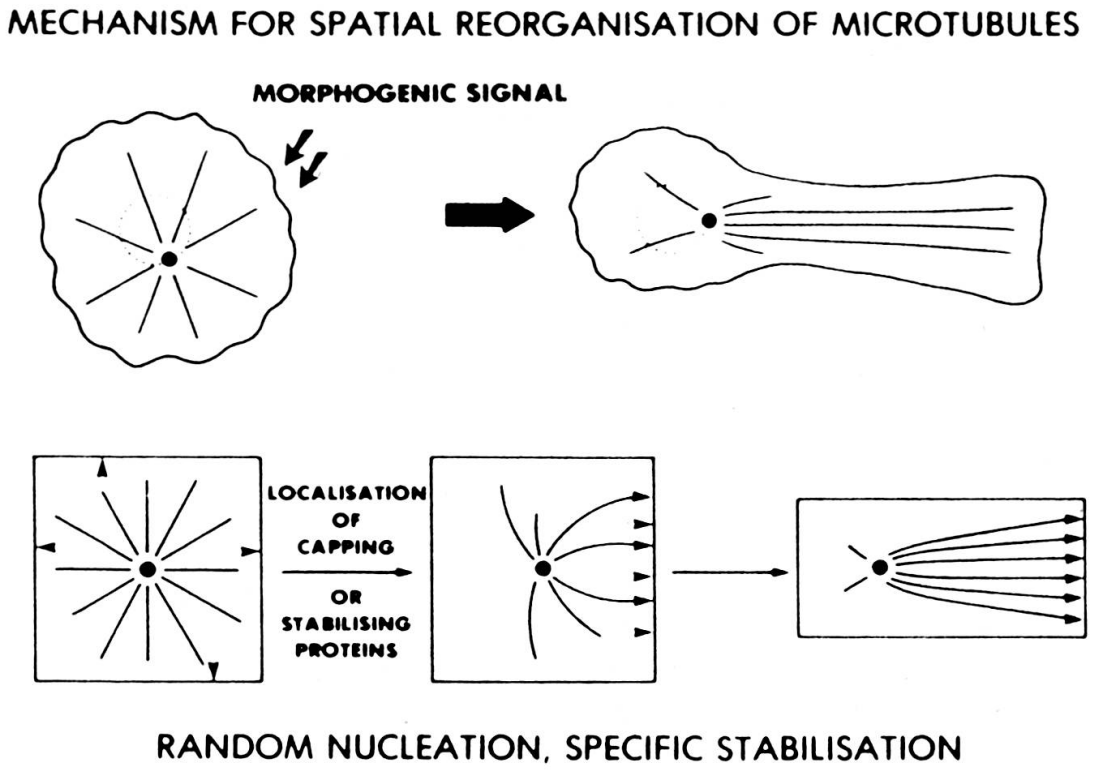


FIG. 16.

Morphogenesis by specific stabilisation. Polarization of cells frequently involves redistribution of microtubules. This is postulated to occur by the local activation of capping or stabilising factors in response to a morphogenetic signal. Microtubules are nucleated in random directions, but those that grow into a specific region of the cell become preferentially stabilised, leading to a polarization of the microtubule distribution. From Mitchison and Kirschner, 1984, authorized by Cold Spring Harbor Symposia, U.S.A.

that contains the centriole pair. Kinetochores are also capable of nucleating microtubules in vitro. The kinetochore in contrast appears to be much less efficient at nucleation in vivo and cannot nucleate below the steady-state concentration. This organelle, unlike the centrosome, does not seem to be designed for nucleation, and perhaps nucleation is not normally an important part of kinetochore function. The kinetochore may interact with microtubules principally by capturing polar microtubules (Witt *et al.*, 1980; Pickett-Heaps *et al.*, 1982). The centrosome is thus clearly designed to nucleate microtubules, with a uniform polarity, and can do so below the concentration where free microtubules are stable. In summary, the half-life of a given microtubule in a closed system such as the cell, and thus the dynamics of the array, depends on the free-dimer concentration, which in turn depends on the number of microtubules present. Increased nucleation capacity will result in increased numbers of microtubules, decreased dimer concentration, and decreased stability of each microtubule.

Data of Mitchison and Kirschner (1984) strongly suggest that the centrosome is the most important nucleating center in mitosis and that the kinetochore has the

ability to capture and cap microtubule ends. Thus, when the nuclear envelope breaks down, the kinetochore is exposed to transiently stable microtubule plus ends, and can capture and cap them. Eventually, enough microtubules will be captured to form the kinetochore fiber, and the polarity of these microtubules will be *plus* ends toward the kinetochore, reflecting the absolute specificity of the centrosome for nucleating with plus ends out. In Mitchison and Kirschner's model "the kinetochore needs have little respect for polarity in its interaction with microtubules, and the polarity of the half-spindle is determined solely by the end specificity of centrosomal nucleation".

Microtubules nucleated at the kinetochore appear first as randomly oriented fragments (De Brabander *et al.*, 1980) and may grow out with random polarity. However, the centrosome may not have the capacity to capture, so that microtubules nucleated at the kinetochore will quickly depolymerize. If the centrosome can capture, it is likely to be specific for the capture of *minus* ends, reflecting the end specificity of its nucleation capacity. The polarity of ATP-dependent movement was determined from the polymerization rate of ends distal to the kinetochore: it was found to be uniformly toward plus ends (Mitchison, 1988).

The formation of the metaphase spindle, a paradigm in cellular morphogenesis, may be partially explicable with reference to the properties of its constituents observed in vitro (Mitchison and Kirschner, 1984). The specificity of the centrosome in nucleating microtubules of uniform polarity defines the polarity of the half-spindle, the capture and cap reaction of the kinetochore stabilize the kinetochore fibers, and the dynamic instability phenomenon implies that the final morphology of the spindle reflects morphogenesis by stabilization rather than by nucleation.

In contrast to the generally accepted older view that chromosomes are passive objects pulled by "traction fibers", Gorbsky *et al.* (1987) have suggested that "the kinetochore is an active participant in generating the motive force that propels the chromosome to the pole".

In another recent attempt to explain the polewards chromosome movement Koshland *et al.* (1988) constructed complexes between isolated chromosomes and microtubules made from purified tubulin. This led these authors to explain the movement of chromosomes towards the "minus" end of microtubule *in vitro* powered and regulated by microtubule depolymerization at the kinetochore. As models proposed for such chromosome-to-pole movement there are: a) walking of translocated ATPase at the kinetochore on the microtubule towards the minus polar end; this ATPase pulls the microtubule towards the kinetochore; b) application of the force on the kinetochore pulling it towards the pole by an elastic element; c) microtubule depolymerization producing force for moving the chromosome to the pole. The question remains of "how can the thermodynamic drive towards microtubule depolymerization be transduced by the kinetochore to provide the force for chromosome movement?" (Koshland *et al.*, 1988).

V. POLAR CELL MOVEMENTS

Polarity appears to regulate the direction of cytoplasmic and cell movements. These movements can be "internal", such as cyclosis in plant cells, or result in a major change in shape or position of the cell. In both aspects, microfilaments and/or microtubules are fundamental to these activities. In most cases, an interaction between proteins such as actin-myosin or dynein-tubulin systems, with the simultaneous involvement of an ATPase, would be the underlying biochemical phenomenon (Condeelis, 1983).

Polarized growth of eukaryotic microorganisms such as fungi has the expected attributes of growth (biosynthesis, especially of new hyphal wall material) but also some of the attributes of motility, i.e. some protoplasm, and in extreme cases, all viable material, is moved from its site of synthesis (Carlile, 1980). This effect has something in common with the movement of an amoeba or a myxomycete plasmodium: in one instance empty hyphal walls are left behind, in the other slime from the glycocalyx, and in both protoplasmic streaming is involved.

A. Cytoplasmic movements

Cytoplasmic streaming or *cyclosis* is easily observed in plant cells, in which the cytoplasm is generally reduced to a layer next to the cellulose wall and to fine trabeculae crossing the large central vacuole. Continuous currents can be seen that displace chloroplasts and other cytoplasmic granules. In some plant cells the protoplasmic current can be initiated by chemicals (*chemodynesis*) or by light (*photodynesis*). Cyclosis is modified by environmental factors such as temperature, pH, etc.

Eukaryotic microorganisms can achieve far higher localized growth rates than prokaryotes. A hypha, for example, can increase in length far more rapidly than can a bacterial cell; the hyphae of *Neurospora crassa* can grow at 100 μm min^{-1} and the sporangiophores of *Phycomyces blakesleeanus* at 60 μm min^{-1}. Materials are transported by protoplasmic streaming to the hyphal apex where extension occurs from a growth zone which may extend for several millimeters behind the apex (Trinci, 1978*a*, *b*).

In microplasmodia of the acellular slime mold *Physarum*, the motive force for protoplasmic streaming is generated in conjunction with the associated contractile apparatus (Wohlfarth-Bottermann, 1975). The actual motive force is provided by peripheral microfilaments situated in the region between the stationary ectoplasm and the moving endoplasm. Cortical microfilaments have mainly been involved in

motive force generation for cytoplasmic streaming activity rather than the fibrillar system extending through the matrix (Brix *et al.,* 1987). Bundles of actin microfilaments all with the same polarity, i.e. with the barbed end anchored in the membrane, form cables. The polarity of their actin filaments is such that the movement of myosin filaments along them could produce the observed cytoplasmic streaming (Fulton, 1984).

The cylindrical cells of the green algae *Nitella* and *Chara* are enormous, being 2 cm to 5 cm in length. These giant, multinucleated cells provide the best examples of cytoplasmic streaming: a continuous ribbon of cytoplasm streams along a gentle helical path down one side of each cell and back across the other side in an endless belt. The streaming cytoplasm moves in only one direction, at speeds of up to 75 μm per second, sweeping internal membranes, mitochondria, nuclei, and cytosol around and around the cell.

Cytoplasmic streaming has been observed to occur at two different velocities: chloroplasts are transported at 1-2 μm s^{-1} and nuclei move in "head-streamed bands" at 3-11 μm s^{-1}. The streamings also differ in the mechanisms of force generation (Koop and Kiermayer, 1980). The cytoplasmic streaming in *Nitella* has been shown by Kamiya (1981) to be driven by an actin and myosin system. The actin cables which exist on the cytoplasmic surface of the *Nitella* membrane are oriented as expected for myosin-driven movements, although there is yet no demonstration that a *Nitella* myosin drives cytoplasmic streaming (Warrick and Spudich, 1987). In Characean algae, direction of streaming has been ascribed to a shearing force generated between the organelle-bound myosin and the actin cable according to the model proposed by Williamson (1975).

In lower animals such as *Paramecium,* similar but slower movements are seen that displace the digestive vacuoles from the site of ingestion to the site of excretion. In many cells of higher animals, particularly in tissue cultures, intracellular movements can also be seen.

B. Cell movements

1. *Cilia-flagella*

Microorganisms may possess cilia or flagella which act as locomotion organelles. The distinction between ciliary and flagellar movement is not always clear-cut, cilia typically causing movement of liquid by beating at right angles to their long axis, while flagella move along the whole length of their axis. Flagella that are located only at one or both poles of the bacterial cell are called polar and bacteria so-called *mono*polar or *bi*polar flagellates. An organism carrying a single polar flagellum would be monopolar monotrichous, while one with a tuft of such flagella would be

monopolar polytrichous or of the lophotrichous type. By contrast, *Spirilla* are bipolar polytrichous or of the amphitrichous type.

The bacterial flagellum consists of three morphological parts: the filament (fibers of the protein flagellin), the hook and the basal body which binds the hook and filament to the cytoplasmic membrane. Some non-flagellated bacteria of the genus *Spirillum* have a polar plate internal to the cytoplasmic membrane.

Reconstitution experiments *in vitro* with *Salmonella* flagella have shown that the flagellar filaments have structural polarity and that they grow by a sequential orderly polymerization of flagellin molecules at a structurally defined end, called the T-end, of each flagellar filament (Asakura *et al.*, 1968). The T-end corresponds to the distal end of a flagellum attached to the cell. Therefore, if flagellar growth *in vivo* is homologous with that *in vitro,* it must take place at the tip.

Bacterial flagella have been found to be assembled by the addition of newly synthesized flagellin monomers onto growing flagella at the tips of the organelles. The assembling process is inverse in non-flagellar, filamentous appendages called fimbriae or pili which are present on every species of Gram$^-$ bacteria and are responsible for the adherence of bacteria to a variety of eukaryotic cell surfaces. According to Lowe *et al.* (1987), the pattern of labelling with gold particles on individual fimbrial organelles indicated morphologically that newly synthesized subunits are added to a growing organelle at its base (Emerson *et al.*, 1970).

Iino's (1969) experiments showed that *Salmonella* flagella grow at their free ends. This agreed with the polarity of flagellar growth *in vitro* (Asakura *et al.*, 1968). Thus, the basic molecular mechanism of flagellar growth *in vivo* may be homologous with that of polymerization of flagellin molecules into flagella *in vitro.*

As for the proximal part of a flagellum which ends as a structure called a "hook", it is connected to a spherical granule, called a "basal granule" (Iterson *et al.*, 1967). These basal structures are presumed to be responsible for the synthesis of flagellin and initiation of flagellar formation as well as flagellar movement.

Movements of flagella are best explained, at least for polar flagellates, according to the theory put forward in 1969 by Vaituzis and Doetsch that the filament of a flagellum is an inert, relatively rigid structure that owes its wavelike appearance to the tertiary structure of the protein; the motion would be initiated by a wobbling action of the basal disc that is embedded in the cytoplasmic membrane (see p. 140).

Among *non*-flagellated bacteria, *Spirochaeta* move by producing helical waves along their length as well as bending, curling and lashing; the motor organelles consist of a single or bundle of fibril(s) attached to both ends and lying under the surface. Possibly related to that type of movement, cell surface of *Spirochaeta* is electrically polarized (James, 1957), contrarily to most other individual organisms in which electric negative charges are distributed evenly and produce there the so-called zeta potential.

Cilia and flagella of eukaryotes are well suited systems for studies of synthesis and assembly of specific proteins. One of the most extensively studied systems is that of *Chlamydomonas* with its model of monopolar flagellar regeneration and its numerous mutants showing aberrant control of flagellar length, assembly, motion, stability and regeneration (Lefebvre and Rosenbaum, 1986). The two ends of flagellar microtubules demonstrate different assembly in vitro at different rates (Allen and Borisy, 1974; Binder *et al.*, 1975). This polarity is unravelled with a method in which partial microtubule sheets are assembled onto preformed microtubules to determine the intrinsic polarity (Euteneuer and McIntosh, 1981*a*). The partial microtubules form hooks whose orientation indicates the polarity of the template microtubules (Lefebvre and Rosenbaum, 1986).

2. *Gliding movements*

There are active even though slow movements in contact with a solid substratum which are carried out by microorganisms such as the primitive Mycoplasmas, and a number of bacteria from Cyanobacteria (blue-green "Algae") to Myxobacteria as well as by certain Algae (diatoms) and Protozoa. The mechanism of this gliding is not yet clearly understood. In Cyanobacteria several alternative models have been proposed (Häder, 1987) to the early hypothesis of an asymmetric secretion of slime.

Mycoplasmas are considered as the smallest prokaryotes possessing very limited genetic information even though endowed with gliding motility. In contrast to the gliding bacteria, the gliding mycoplasmas have a distinct antero-posterior axis; their leading anterior end is distinguishable by a specialized cytoskeletal structure of actin-like proteins (Bredt, 1979; Göbel *et al.*, 1981) as possible locomotor apparatus fueled by cell derived energy. Mycoplasma gliding would therefore insure unidirectional cell movement by an active form of surface translocation rather than a biased Brownian motion (Rosengarten and Kirchhoff, 1988).

Bacterial gliding motility appears to be dependent on the establishment of a transmembrane potential and possible metabolic pathway (cyanide-insensitive) for maintaining it and motility in *Flexibacter* have been proposed (Duxbury *et al.*, 1980). Any depolymerization of the membrane (by 2,4-DNP, CCCP, etc.) results in a cessation of motility. For gliding movements, the energy source for the motor is not ATP but protons circulation (Harold, 1977). Interestingly, the presence of this proton force near one end of the cell would create a potential difference along the long axis of the cell additional to that existing across the plasma membrane and the ensuing polarization may provide a triggering mechanism generating rhythmical oscillations of the bacterium. This requirement for a suitable transmembrane potential in gliding bacteria is consistent with a translation of the electrical potential into some form of propulsion mechanical work (Duxbury *et al.*, 1980).

Gliding filaments of the cyanobacterium *Phormidium uncinatum* rotate during forward locomotion. They respond to a sudden decrease in light intensity by a phobic

reversal of movement. The photophobic response leads to a gliding reversal directed by transductional sensory chain which has been biochemically modelled (see V.B.5).

Temporal polarity of motile filaments of blue-green algae might be due to an electrical potential difference between front and rear end (Häder, 1980). Since this potential is a prerequisite for the phobic reversal of movement, it is possible to inhibit photophobic responses by the application of external electrical potentials. Moreover, as gramicidin inhibits photophobic responses in *Phormidium,* "it can be concluded that an ionic gradient exists across the membrane which is cancelled by the ionophore". The physiological polarity of a trichome determines the direction of movement and is manifested in a potential difference in each cell (Fig. 34 in Häder, 1980).

3. *Amoeboid motion (transient polarity)*

In this form of locomotion, the cell changes shape actively, sending forth cytoplasmic projections called *pseudopodia,* into which the protoplasm flows. Although this special form of locomotion can be observed easily in true protozoan amoebae (amebae), it also occurs in numerous other types of cells from the prokaryotic "amoebae" of *Mycoplasma* and the myxamoebae of slime molds to the human leukocytes (diapedesis).

Some amoebae are predominantly *monopodial* (one pseudopodium), therefore presenting transient *mono*polarity. Others may be temporarily or permanently *polypodial* or in a shape of *poly*polarity. In an unconstrained situation, interphase myxamoebae of *Dictyostelium discoideum* moved around randomly in a polypodial or monopodial fashion. There is good evidence that pseudopodial activity and locomotion are two dissociated events (Roos *et al.,* 1986).

The shuttle streaming of endoplasm in plasmodial veins of the slime mold *Physarum polycephalum* follows local hydrostatic pressure alterations in different regions of the plasmodium ("pressure flow"). Hydrostatic pressure gradients result from intrinsic contraction automaticity of cytoplasmic actomyosin in the ectoplasmic wall of the veins. The flowing (i.e. relaxed) endoplasm contains depolymerized actin. In the ectoplasmic wall of the strands, the actomyosin undergoes rhythmic contraction-relaxation cycles accompanied by $G \rightleftharpoons F$ actin transformation and actomyosin fibrillogenesis (during isometric contraction). According to Wohlfarth-Bottermann (1983), ecto-endoplasm transformations involved in plasmodial locomotion can be regarded as a part of the contraction-relaxation cycle of cytoplasmic actomyosin.

The protoplasm of the amoeba has a clear ectoplasm, which expands considerably toward the end of the pseudopodium. The axial endoplasm is surrounded by a "shear zone" where particles move freely. At the advancing end is the hyaline cap and just posterior to it, the "fountain zone", where the axial endoplasm appears

to contract actively and flows below the ectoplasmic tube. At the opposite end is the tail process, also called the *uroid,* and near it the *recruitement zone,* where the endoplasm is recruited from the walls of the ectoplasm in the posterior third of the cell (Giese, 1979; Taylor *et al.,* 1982; Fulton, 1984).

Motile activity in amoeba of cellular slime molds has also been biochemically characterized (Taylor and Condeelis, 1979; Taylor *et al.,* 1982). Actin and myosin were shown to interact to produce amoeboid movement. In aggregating, polarized amoeba, actin was found primarily in the anterior pseudopodium. However, myosin was not always codistributed with actin (Rubino *et al.,* 1984), a puzzling fact with respect to the generally accepted actomyosin theory of amoeboid movement. Indeed, myosin filaments in myxamoebae were found to be primarily localized at the posterior cortex which would thus be the site of motive force production to move the polarized cell (Fukui and Yumura, 1986). Changes in myosin heavy-chain, as well as light-chain phosphorylation occur in *Dictyostelium* cells responding to the chemoattractant cAMP (Berlot *et al.,* 1985). Surprisingly, this myosin has been shown by genetic manipulation experiments to be nonessential for chemotaxis (De Lozanne and Spudich, 1987; Knecht and Loomis, 1988). Pseudopods extension is correlated with the appearance of dense microfilament networks at sites of pseudopod growth. Actin polymerization may constitute one of the driving forces for extension of pseudopods, and nucleation sites regulating polymerization are under the control of chemotaxis receptors (Condeelis, *et al.,* 1988).

In migrating giant amoebae of the *Amoeba proteus* type, the dense filamentous actin-containing cortical layer is always continuous over the entire surface and varies in thickness depending on the cell polarity. At the front of the cell it is thin while in the trailing uroid regions it increases in thickness. Thin actin filaments are in all parts of the cortex, contrarily to thick filaments, probably myosin, which are present mainly in the uroid region. In intermediate regions, "a flow of cortex from the front to the back of the cell is coupled to the plasma membrane and through it to the substratum, where it drives the forward migration of the cell" (Bray and White, 1988).

An important factor in amoeboid motion is adhesion to a solid *support.* An amoeba that floats freely in the liquid medium can emit pseudopodia, but does not progress; only when it adheres to a solid surface does it commence this type of locomotion. This is also known in aquatic Fungi where the zoospores are creeping on solid surface.

In their natural environment, cells crawl over a complex network of molecules known as the extracellular matrix. The matrix consists largely of the protein collagen and various long chain polysaccharides. Included in the network are some specialized large proteins — like fibronectin — that the cell recognizes as it moves along. The cell's attachment is mediated by a receptor for fibronectin in the plasma membrane.

The receptor recognizes fibronectin and binds to it, forming a "foot" that provides the thrust for forward motion.

According to Abercrombie *et al.* (1970), cell movement is achieved by a continuous backward flow of membrane from the leading edge of the cell to its trailing edge. They saw that such a membrane-transfer cycle would thus enable the cell to extend itself forward over the substratum. To explain the mechanism of amoeboid movement, Bignold (1987) has proposed a "membrane ratchet" model which invokes the existence of specific, laterally mobile, transmembranous structures in the cell membrane; such structures are reversibly adhesive for both the contractile apparatus of the cell internally, and the substratum externally. A more recent model for general cell locomotion has been proposed (Fig. 17). The driving force involves a concerted flow of actin filaments associated with the inner face of the plasma membrane: "the movements originate at a specific region at the cell surface — the leading edge of migrating cells or the polar region of a dividing cell — and carry material back over the cell surface to a more proximal position" (Bray and White, 1988).

Receptors are concentrated at the leading edge, as shown by the high concentration of ferritin receptors on the ruffling edge of the cells tested (Bretscher, 1987). A similar distribution was found for transferrin receptors on giant HeLa cells. Non circulating proteins, on the other hand, were found to be distributed more or less at random on the surface. Such experiments imply that exocytosis occurs at the front edge of a motile cell and that this process, directed toward the leading edge, is sufficient to account for the actual forward movement seen in fibroblasts (Bretscher, 1987). Nevertheless, remains the challenging question of what distinguishes the leading edge of a motile cell so that exocytosis takes place here and not elsewhere.

When a positively charged polymer such as polylysine is added to the medium, it reduces the net charge on the surface of the membrane and the membrane contracts. This contraction can be neutralized by washing or by adding a negatively charged polymer such as polyglutamic acid or heparin. All these experiments are carried out in the absence of bivalent cations such as calcium or magnesium; but if calcium or magnesium are present it is possible to produce contraction also with negatively charged substances. For example, polyglutamic acid or heparin in the presence of calcium or magnesium will cause contraction and more important still, ATP in the presence of magnesium will also cause contraction. In the case when the net charge is reduced by a negatively charged substance associated through calcium or magnesium with the cell surface, we are presumably dealing with a bridge between the two, formed by the bivalent calcium or magnesium ion. The important point here is that the net charge on the surface of the isolated nuclear membranes is acting in very much the same way as the net charge acts between cells: when there is a high net charge, the charges repel each other strongly and cause the membrane to expand; when the charge is reduced the repulsive force at the surface (that is, tangentially along the surface) is reduced and the membrane contracts. This effect has been

demonstrated to be related to actual cellular locomotion and to transmembrane potentials. In *Amoeba proteus* a local injection of polylysine with a micropipette near the leading pseudopodium causes immediate contraction and that region of the cytoplasm begins to flow in the reverse direction.

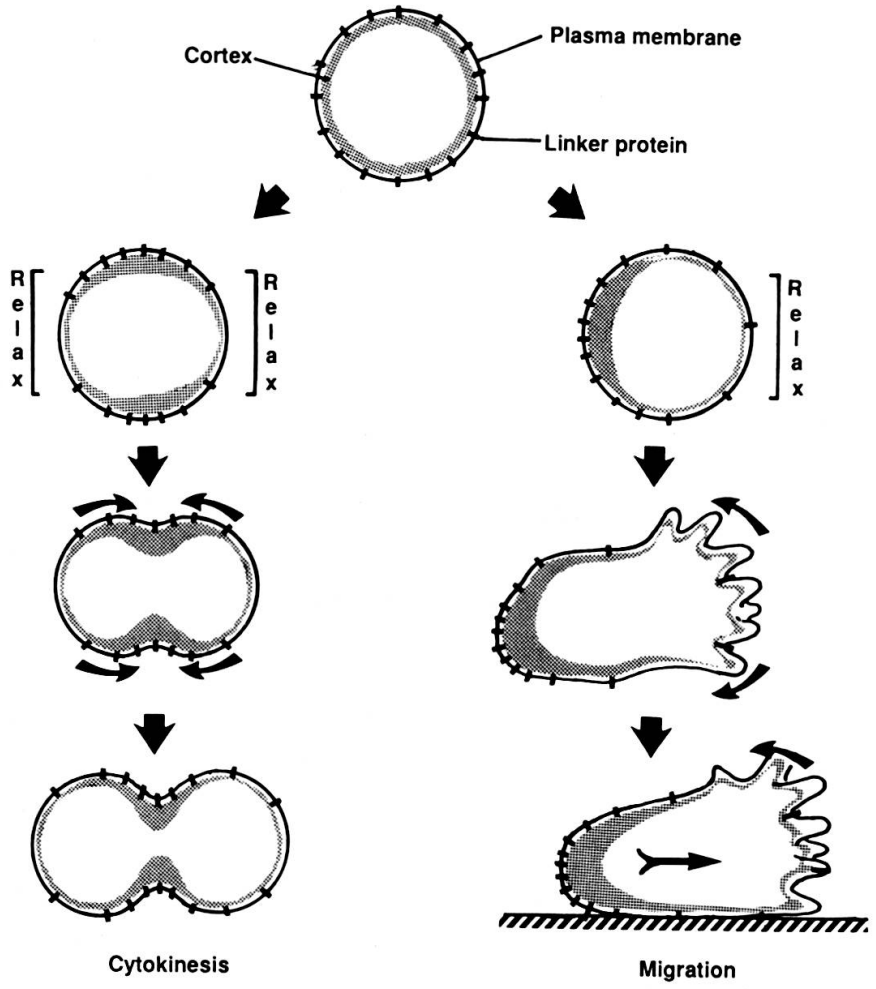


FIG. 17.

Analogy between cortical movements during cytokinesis and cell migration. In this diagram the cortex is represented in cross section, lying close to the inner face of the plasma membrane to which it is attached through special linker proteins. The linker proteins are connected with molecules on the external surface of the cell, such as those concerned with adhesion to a substratum. In *cytokinetic* cell, relaxation at the polar regions (or, what is equivalent for the present purposes, contraction at the equator) leads to the accumulation of actin and associated protein in a band around the equator of the cell. In *migrating* cell, only one region is relaxed and cortical components are pulled in a unidirectional flow toward the region of greatest tension. Here components of the cortex are dispersed and carried forward through the cytoplasm to complete the cycle. Although the flow continues in a cell suspended in fluid medium, contact with a solid substratum permits attachments to be formed with the cortical linker proteins; the cortical flow will consequently drive the cell forward, as shown. Bray and White, Fig. 2, *Science,* 239: 883 (1987), with permission.

Electrical polarity can also be demonstrated with amoebae, the regions of advancing pseudopodia having high negative charge and the tail having a lower

charge. There is a striking correlation between amoeba's morphology changes produced by spontaneous reversals of cytoplasmic streaming polarity and changes in transcellular current patterns. The giant amoeba *Chaos chaos* drives a steady current (0.1 $\mu m/cm^2$), probably carried by Ca^{2+}, into its tail and out its pseudopods (Nuccitelli *et al.,* 1977). The reversals of polarity are always *preceded* by a change in the transcellular current such that the region of largest inward current becomes the new tail (Nuccitelli, 1983).

In an oriented process known as diapedesis, leukocytes can wander out of the blood vessels by active amoeboid motion and progress toward the focus of infection (Schiffmann, 1982). With their dynamic elastic behavior, erythrocytes provide a simple viscoelastic model of cellular elongation which could microscopically be recorded. When an erythrocyte cell suspension was placed between two electrodes with sharp edges, it led to the accumulation of opposite charges at the two ends of the cell opposing the electrodes (Engelhardt *et al.,* 1984). Due to this so-called Maxwell-Wagner polarization, each cell behaved as an electric dipole which could be coupled to the electric field (Pohl, 1978).

When a fibroblast from a tissue culture is in motion, it is often roughly triangular in top view: one side of the triangle forms the front, or leading edge, and the cell tapers off behind to a narrow trailing edge. This activity does not follow a predictable pattern. One region may briefly extend forward over the substratum; then, when the activity subsides, another region along the leading edge may spread out. The sequence is repeated until the cell has advanced along its entire front (Fulton, 1984).

When single kidney cells are seeded out in a blood plasma medium, each cell secretes an as yet unidentified liquid product — perhaps primitive urine — a large vacuole which indents the nucleus and eventually is extruded from the cell. Each isolated cell thus forms a liquid puddle around it in the plasma clot; however, the point of extrusion is in each cell quite haphazard. As soon as the dispersed cells combine into groups, as a result of accidental collision and homotypic aggregation, they begin to polarize each other, each acquiring a basal apical axis, with secretion no longer being arbitrary, but all cells now extruding at their new apical surface — that is, towards the inside — which leads to the physical result of, at first, a vesicle. As soon as a certain critical mass of cells has developed, the lumen is closed off. This deforms the structure further, the nucleus moves towards the polar axis and elongates, and if mesenchymal (stromal) cells are in the vicinity, a basement membrane is formed, the mechanical properties of which confine the total diameter of the structure to a standard size. That leads to a cylindrical extension, which may branch whenever the growing mass exceeds a certain threshold value.

4. *Amoeba-flagellate reversible transformation*

In the acellular slime mold *Physarum polycephalum,* haploid cells transform reversibly between biflagellated swarm cells and amoeboid cells depending on

environmental conditions. Events which take place during these changes include "de novo" formation or disintegration of flagella, redistribution of organelles and morphological changes between a non-polarized amoeba and an elongated, polarized cell with a distinct shape (Goodman, 1982). This strongly suggested the involvement of changes in cytoskeletal structures, as cytochemically unravelled by Uyeda and Furuya (1985). The microtubular cytoskeleton of *Physarum* flagellates was found to be formed by some modification into a cone structure of microtubules already present in amoeba. Microfilaments were detected in both stages of the slime mold, more peripherally with extrusions toward pseudopods in the amoebae, as a dorsal ridge in the flagellates. This last structure apparently coincided spatially with one of the bundles of microtubules (Uyeda and Furuya, 1985). According to Schuster's model (1965) for amoeba-to-flagellate transformation, "once the cell acquired an elongated shape, this was maintained thereafter". In their contrasting model, Uyeda and Furuya (1985) have proposed that "after a cell has become elongated and polarized, accompanying extrusion of the flagellar cone, it temporarily rounds up into a disk-like shape, while retaining its polarity".

Free-living amoebae of the protozoan genus *Naegleria* display a predominantly monopodial "limax" pattern of locomotion. Enflagellation normally occurs when amoebae are subjected to nutritional deprivation (Marciano-Cabral, 1988). Oppositely to the myxamoebae of *Physarum,* interphase *Naegleria* amoebae were found to lack of microtubular structures (Fulton, 1977) and, thus, cytoskeletal microtubules of its flagellates were suggested to be formed completely "de novo" together with flagella and basal bodies (Walsch, 1984).

As an amoeba, *N. gruberi* possesses no fixed polarity, forming pseudopodia in all directions. Within minutes after exposure to an inducing signal (low ionic strength) pseudopodial outgrowth becomes polarized; only one pseudopodium appears at one end and exhibits directed movement. Shortly thereafter fine projections appear at the posterior end and transform into a flagellum. This latter region now becomes the anterior, leading end, reversing the polarity of the cell.

Polarity of the cell is probably determined by factors controlling the position of the basal granules. In most cells these granules preexist or are derived from centrioles, but in *Naegleria* no preexisting centrioles or basal granules have been seen. This implies that the basal granule arises de novo by mechanisms still unknown and becomes fixed within the cortex at the posterior end. It is not clear whether the granule forms randomly in any region of the cell and migrates to the posterior end, or whether it is assembled in its final position. It does become fixed in the region of an amoeboid cell that is most rigid and under a state of contraction.

The change between the two motility forms of the amoeba *Naegleria gruberi* has been further studied by Lai *et al.* (1984) as a Yin-Yang, in Chinese thought the two complementary forces or principles that make up all aspects and phenomena of life (Encyclopedia Britannica, 1980). As explained by Lai *et al.* (1984). "This is

consistent with the current understanding of cell shape and motility as it applies to this differentiation, but it is also simplistic. Although no cytoplasmic microtubules have been observed in *Naegleria* amoebae, the amoebae use tubulin for mitosis. Therefore the capacity for amoeboid motility remains, albeit latent, in flagellates."

The cells change from amoebae with an actin-based motility system to flagellates with a tubulin-based system. Thus, they alternate between two fundamental motility forms. The actin-based motility system remains latent in the flagellates, but can be reactivated almost instantly by suitable stimuli (Fulton, 1977). In addition to the microtubules of the two flagella and basal bodies, the flagellates have a cage of microtubules just beneath the cell surface (Fulton, 1977; Walsh, 1984). The tubulin-based system is more impressive than the actin-based system in the sense that flagellates swim about 100 times as fast as amoebae walk. According to Lai *et al.* (1984), during the time that the cells are changing from an actin based to a tubulin-based motility, actin, the major protein of both amoebae and flagellates, is neither synthesized nor degraded, whereas tubulin for the flagella is synthesized de novo. The abundance of translatable mRNAs for tubulin and actin rise and fall in parallel with these changes.

The amoeba-to-flagellate transformation appears to be both directly and indirectly controlled by calcium (Marciano-Cabral, 1988). Its inhibition by the ionophore A23187 reveals a direct role of calcium while its inhibition by trifluoperazine is indirectly mediated by the calcium-regulatory protein calmodulin, itself controlled by cyclic nucleotide levels (Schuster and Twomey, 1983).

5. *Taxis*

a) *Chemotaxis*

Chemotaxis can be defined as the property of several substances to influence cell motion by attracting or repelling the cells or, in short, cell motion directed by external chemical gradients. Most relevant among the issues recently raised by Devreotes and Zigmond (1988) about "how do cells accomplish chemotaxis?" there is that of "how is the directional response achieved?" Other unsolved questions are "Do chemoattractant receptors move in the plane of the membrane? What localizes components of the cytoskeleton in a locomoting cell?"

Chemotaxis is not only most clearly displayed by eukaryotic models — slime mold and leukocytes — but also by many prokaryotes. Among mycoplasmas, helical spiroplasmas (Daniels and Longland, 1984; Daniels *et al.*, 1980) display a motion that appears to be a rotation around the helix and a flexing of the cell body (Davis and Worley, 1973). Using the motility track technique Kirchhoff *et al.* (1987) were able to demonstrate chemotactic behavior of a gliding mycoplasma that is moving very fast. Different opinions exist about chemotactic behavior of gliding, flagellated bacteria (Adler, 1975). This behavior has been described by some authors (Ho and

McCurdy, 1979), but negated by others (Dworkin and Eide, 1983) and it should be determined whether the use of the simple motility track technique will end this controversial discussion. In chemotactic bacteria *spatial* concentration differences of attractants can be translated into changes of concentration with time. Their *temporal* response has been found to be based on adaptation due to the methylation of a set of membrane proteins (Goy *et al.*, 1977).

Bacteria have sensory receptors — chemoreceptors — that detect the attractants per se; the first identified at the molecular level, by using deficient mutants, was the one for galactose which turned to be the galactose binding protein (Adler, 1980). Bacterial chemoreceptors serve a *dual* function: transport and chemotaxis; chemoreceptors being located in the cell surface, chemicals can serve as attractants without entering the cell. The control of the frequency of tumbling is the central feature of bacterial chemotaxis; its control applies not only to spatial gradients but also to temporal ones (Koshland's group, 1972-73, in Adler, 1980). Between the receptors of stimuli and the effectors is a system for integrating the sensory information and transmitting a message to the flagella. The system of sensory transduction somehow involves methylation of membrane proteins (Adler, 1980).

Proteins regulated chemotactic responses in *E. coli* are methyl-accepting chemotaxis proteins or MCPs (see Saier and Jacobson, 1984). Attractants stimulate methylation of one of these proteins (by a methyltransferase) while repellents cause demethylation (by a methylesterase). By their autophosphorylation, these proteins initiate a cascade of protein (histidine-) phosphorylation which may be important in the signal transduction pathway of bacterial chemotaxis (Hess *et al.*, 1988). Sensory information for chemotaxis is gathered by a set of transmembrane receptors, integrated and transmitted to the flagellar "switch" which regulates the direction of rotation of flagellar filaments in response to extracellular stimuli. The MCPs therefore play the decisive role in the conversion of sensory stimulus into behavioral, positive or negative polar response modulated by flagellar rotation.

The energy for rotation of bacterial flagella was shown not to be the ATP (unlike eukaryotic flagella), but rather the proton motive force (Adler's group, 1974 and Berg's group, 1977, in Adler, 1980). Prokaryotic flagella rotate at their base where is located the rotary motor comprising rings, thought to act as stators and rotors for the proton motor. Rotation of these ring structures with respect to another could then generate the torque necessary to spin the flagellar filament of flagellin. It is believed that the proton electrochemical gradient provides the energy for flagellar rotation (see Lehninger, 1982; Saier and Jacobson, 1984).

In the cellular slime mold *Dictyostelium discoideum,* amoebae are aggregated by a sophisticated chemotactic system (Bonner, 1974). Assessment of the directional role of a cyclic AMP gradient has provided one of the best understood examples of signal transduction across a biological membrane. Amoebae migrating away from the "source" of cyclic AMP in the spatial gradient extend lateral pseudopods in a

polarized fashion (McRobbie, 1986). Even though a spatial mechanism has been involved in assessing spatial gradient of the chemoattractant, a temporal gradient of cyclic AMP generated by an amoeba moving through the spatial gradient might play a major role in chemotaxis (Varnum-Finney *et al.*, 1987). In a recently proposed model (Klein *et al.*, 1988), the cyclic AMP receptor — rhodopsin-like, hydrophobic residues-rich — crosses the amoebal membrane with a serine-rich cytoplasmic carboxyl terminus, the proposed site of ligand-induced receptor phosphorylation.

Amoebae have two possibilities of sensing a spatial gradient of chemical attractant: they can measure concentration differences over their surfaces, or they can extend pseudopods into different directions using them as sensors. During its extension a pseudopod can translate spatial concentration differences into changes of concentration with time. It is an open question if, in the chemotactic response of amoeboid cells, such a temporal mechanism is involved. To solve this question for amoebae, chemical mediators of the chemotactic response have been searched to study their temporal behaviour after addition of attractant to living cells. This search for intracellular mediators of the chemotactic response has been focussed on changes which occur within the first few seconds after the stimulation of cells by cyclic AMP or other attractants like folic acid (pterins). A strong polarization could prevent the cells of *D. discoideum* from reorienting in a reversed gradient: cells attracted to a pipette-imposed gradient can reverse reorientation when the pipette is repositioned at the back (Loomis, 1982).

Interference of colchicine with chemotaxis (Oliver and Berlin, 1982) has led to consider that arrays of microtubules, organized from the centrosome, might provide direction to cell movement (Schliwa *et al.*, 1983). However, colchicine-treated polymorphonuclear leukocytes viewed microscopically still point in the right direction and, although they show wider angles of turn than untreated cells, they continue to move toward the chemotactic source.

In the ciliate Protozoa of the genus *Paramecium,* the "avoidance" reaction involves a quick change in the direction of ciliary beating. This reaction is critically dependent on the presence of Ca^{2+} ions in the medium and dependent on local intracellular concentration of this ion (excess of 10^{-6} M) influx of Ca^{2+} down its electrochemical gradient causes membrane depolarization, thus provoking the avoidance reaction by ciliary reversal and transient opening of Ca^{2+} channels in the plasma membrane covering the cilia (see Saier and Jacobson, 1984). By contrast, hyperpolarization of the cell membrane resulting from K^+ cell outflow provokes an escape behavioral response in *Paramecium.* Mechanically responsive ion channels are therefore differentially localized in the cell membrane and, as further stated by Saier and Jacobson (1984), "since an anterior stimulus elicits a response opposite to that resulting from a posterior stimulus (i.e. depolarization versus hyperpolarization), and channels of different specificities are initially activated in each case (Ca^{2+} versus K^+), there must be spatial localization of the two receptor channels at

opposite ends of the cell''. Both Ca^{2+} and K^+ channels exhibit the classic voltage-dependence (see IV.B.2d) and their functionings have been compared in several behavioral mutants in *Paramecium* (see Saier and Jacobson, 1984). Recently, the deficient Ca^{2+}-dependent K^+ current mutants called pantophobiacs could be corrected by the microinjection of wild type *Paramecium* calmodulin (Hinrichsen *et al.*, 1986).

Leukocytes are also polarized in their response to changes in chemotactic gradients: when the direction of a spatial gradient is reversed, new pseudopods extend from the front of most leukocytes (Zigmond *et al.*, 1981).

b) *Phototaxis*

Phototaxis is an orientation depending on the direction of the incoming light rays. The red *Halobacterium halobium* can use the light as a signal for orienting itself in the environment through a photoreceptor pigment, bacteriorhodopsin. This retinyl pigment (Häder, 1987) is concentrated in the cell membrane at particular regions of this so-called purple membrane. Upon light absorption, bacteriorhodopsin is deprotonized; this exchange of protons is a vectorial process and the resulting electrochemical H^+ gradient can be used by the cell in different ways such as the synthesis of ATP and the proton-driven pumping of Na^+ ions out of the cell. If the light intensity is suddenly increased or decreased, the halobacterial cell can perform a typical photophobic response (reversed swimming direction). Thus, bacteriorhodopsin is responsible for a dual use of light, both as source of energy and signal for behaviour (Haupt, 1980).

Probably Cyanobacteria are the only prokaryotes capable of a true phototactic orientation (Häder, 1987). The oriented movement takes the form of positive phototactism (toward the light source) or negative phototactism (away from the light source). The two families of *Oscillatoriaceae* and *Nostocaceae* differ in the mechanisms of phototactic orientation. A model for the sensory transduction chain powering the motor of the photophobic gliding *Phormidium (Oscillatoriaceae)* has been proposed by Häder (1982). A proton gradient built up in light through the photosynthetic electron transport chain causes a hyperpolarization in the cytoplasm. When the front cells enter a dark field, this proton gradient breaks down which causes depolarization. The small electrical potential generated between the two morphologically equal ends of the filament — front end more negative than rear end — is supposed to open voltage-dependent Ca^{2+} specific channels in the plasmalemma. This initial small potential change causes voltage-dependent Ca^{2+} channels to open and allow a transient massive Ca^{2+} influx. The resulting large electrical potential change reverses the potential difference between the front and rear ends, which eventually reverses the direction of movement (Häder, 1987). A partially different mechanism for the sensory transduction in Cyanobacteria has been proposed by

Glagolev's group (Gabai and Glagolev, 1985) which implies redox sensing through plastoquinone. From these models of photosensory behaviour in prokaryotes it can at least be concluded that motile filaments of blue-green algae show a temporal polarity which might be due to an electrical potential difference between front and rear end. Consequently, the photophobic reversal of their movements can be inhibited by the application of external electrical potential. The following scheme, proposed by Haupt (1980) summarizes such sensory transduction of the photophobic response of a *Phormidium* trichome:

Light STIMULUS dI/dt ---► TRANSDUCTIONAL changes in: electron flow rate ---► plastoquinone ---► proton transport across thylakoid and plasma membranes ---► electrical polarity of cell ---► reversal of anterior-posterior cell polarity ---► amplification by cell-to-cell transfer ---► cell polarity controls direction of motor activity ---► RESPONSE: reversal of gliding movement.

The gliding flagellate *Euglena gracilis* shows positive and negative phototaxis depending on the light intensity and implicating a flavin-like signal. Since phototactic orientation can be induced in some cases by very low light intensities, a signal amplification by gated cation fluxes needs to be incorporated in the sensory transduction chain (Doughty and Diehn, 1982). Ca^{2+} currents generated by light stimulus also influence the motion of flagella and thereby a positive phototaxis in the green algal-flagellate *Chlamydomonas.*

Dictyostelium discoideum myxamoebae show a positive phototaxis in low light intensity. The direction of light was detected by a comparison between the signal strengths at a multitude of receptor sites around the circumferences of the myxamoebae. A low light beam focussed onto the periphery of a cell induced a reorganization of its internal polarity and movement in the direction of the microbeam (Häder and Burkart, 1983).

Phototactic behavior in *Physarum polycephalum* has been discussed by Mori *et al.* (1986) in terms of bifurcation in spatio-temporal organization in a self-organizing system. Twice more ATP was measured in the front than in the rear of a migrating plasmodium. This polar pattern became unstable, and a new wavy pattern appeared by stimulating a local frontal part with blue light.

In amoeba, transient polarity can also be induced by lateral incident light (Lazowski, 1986). The negative phototactic reaction of *Amoeba proteus* is related to a semi-permanent organization of its cell. Posterior part of the illuminated cell is developed on the more irradiated side, anterior part with advancing pseudopods on the shadowed side. In order to minimize the body area direction, exposed to light, amoeba turns its body to a parallel position in respect to light direction, or it produces the new pseudopods on the shadowed cell side. Then, it gradually orients its body along the light direction, as the leading pseudopod advances. The mechanism underlying the light-induced functional polarity of the amoeba appears to involve

intact membranes as suggested by the disturbing effects of different drugs such as DMSO, digitonin and saponin on the light-induced cell polarity (Lazowski, 1986).

6. *Structural basis of directionality*

The apparent relationship between cell polarity and the position of the centrosome has been postulated a century ago by van Beneden (1883). It received support from recent experimental studies that correlate shifts in centrosome position with changes in the cell axis and/or changes in the direction of cell locomotion. Even though such a correlation between cell polarity and centrosome position could not be established in all instances (Oliver and Berlin, 1982). These and other observations have led to the widely held belief that cell polarity and the direction of cell locomotion are determined by the centrosome, which transmits the necessary information to the rest of the cytoskeleton by way of the microtubules associated with it. It needs to be emphasized, however, that, whereas these studies present evidence for a positive correlation between centrosome position and the establishment of a cell axis or the direction of cell locomotion, it is difficult to determine cause and effect. This question is central to the issue of cell polarity during directional locomotion, but it cannot be resolved on the basis of the available evidence.

Mechanisms required for cell locomotion to occur are: a) intracellular force-generation involving the cytoskeleton; b) polarity-determination involving the insertion of new membrane mass into the leading edge; c) adhesive interactions of the cell surface with the substratum. The insertion of new membrane mass into the leading edge implies an equivalent rate of retrieval of old membrane mass towards the rear of the motile cell. Required for this directed locomotory process is that "ordinary membrane recycling be polarized, i.e. that at least some of the processes of membrane insertion and retrieval (recycling) that normally occur *uniformly* over the surface of a nonmotile cell, be *polarized* so that insertion always occurs at the leading edge and retrieval towards the rear" (Singer and Kupfer, 1986).

Focal adhesions usually form within or just behind the ruffling leading edge of motile cells. A microspike or bundle of radially-oriented filaments of actin precedes the formation of these adhesions. According to Burridge *et al.* (1988) "Talin, but not vinculin, has been detected at the leading edge and is concentrated at the tips of the distal portion of the precursor (Izzard, 1988) where microfilaments terminate in an electron dense patch of material associated with the plasmalemma (Small, 1981)".

Euteneuer and Schliwa (1985*a*) have proposed that the cell cortex influences the position of the centrosome/microtubule complex. This interaction, whose molecular basis remains to be determined, also takes place in polar migratory cells, where the cell cortex, notably extending anterior lamellipod, plays a dominant role. As commented by these authors, the proposed dominance of the cortex, however, does not

mean that microtubules are dispensable and do not take part in directional locomotion of many cell types, perhaps except fish epidermal keratocytes; these last cells appear to be "permanently polarized and therefore may circumvent the need to achieve the polarization that is required by an amorphous cell in order for it to migrate" (Singer and Kupfer, 1986). In keratocytes, the emphasis thus appears to be shifted from a cell component — microtubules — that sets up polarity to an element that helps reinforce polar organization, set up by the cell cortex. Trinkaus (1984) discusses a scenario where microtubules become passively aligned by the cortex, help stabilize cortically determined asymmetry, and thus, in a process of mutual reinforcement, establish cell polarity. In this way the centrosome-aster complex tends to exert a constraining influence and serves to stabilize the bulk of the cell.

The polarizing influence from basal granules is also expressed as a pattern of oriented cilia beating synchronously in waves that direct movement of a ciliated cell. In this way polarity at the molecular level is transformed into organismic asymmetries.

Activation of the cell cortex by chemoattractants (Schliwa *et al.,* 1982) induces cell spreading that is likely to exert a force on the microtubules extending into the peripheral filament network, thus pulling the microtubule aster apart and leading to two, occasionally three, independent asters. As long as the cortex remains active, this condition is maintained. It is reversed upon return to a normal migratory morphology (Schliwa *et al.,* 1982) or experimental disruption of the actin network.

Many cell types are capable of persistent, directional locomotion in the absence of known external stimuli such as chemical signals, substrate properties, or electric fields. Essentially all these locomoting cells possess an external polar organization characterized by the presence of an anterior lamellipod of variable shape and complexity, and a posterior uropod or tail from which retraction fibers may extend. This external asymmetry seems to be complemented in many instances by an internal axis defined by the position of two prominent cell organelles, namely, the nucleus and the centrosome/microtubule complex. The finding of an apparent relationship between cell polarity and the position of the centrosome, postulated a century ago, received support from recent experimental studies that correlate shifts in centrosome position with changes in the cell axis and/or changes in the direction of cell locomotion. Even though such a correlation between cell polarity and centrosome position could not be established in all instances, these and other observations have led to the widely held belief that cell polarity and direction of cell locomotion are determined by the centrosome which transmits the necessary information to the rest of the cytoskeleton by way of the microtubules associated with it.

The leading edge of migrating cells is internally propelled by force-generating systems that are components of the cytoskeleton: cytochalasin B inhibits not only directional migration but all protrusive activity (blebbing). As the primary effect of this drug is to "cap" the barbed ends of F-actin filaments (see IV.E.1), a role for

actin in cell migration is strongly implied by these findings (Singer and Kupfer, 1986). The possible relationship between cell motility and mitochondrial membrane potential has been explored in epithelial cells in culture and mitochondria of the leading edge found to have a higher potential than those located elsewhere (Johnson *et al.,* 1981; Chen, 1988).

In migrating cells, the cytoplasmic vesicular structures and their associated microtubules are more or less oriented in front of the nucleus and face the average direction of motion. Serial sectioning was required to demonstrate such cell polarization for polymorphonuclear leukocytes migrating along a chemotactic gradient (Malech *et al.,* 1977). In fibroblasts migrating out of chick heart explants, mitochondria were highly clustering near the nucleus while they were dispersed more evenly when the cells came to rest in confluent growth (Parsons, 1986). This structural polarization of cells in motion was confirmed in a computer-aided three-dimensional graphics reconstruction of serial thin sections of migrating fetal monkey neurons (Parsons, 1986) and further work is now being directed at quantitating the polarization of organelles in moving cells.

VI. POLAR CELL GROWTH

Living matter expands by oriented growth and should fundamentally request mediation of polarizing processes. As presented in chapter V, certain amoeboid cells which apparently have no visible morphological polarity and could thus be considered as *a*polar, emit pseudopods endowed with reversible mono- or polypolarity. Spherical forms, such as coccoid bacteria and pleurococcoid green algae also exhibit polarity in certain phases of their developmental cycle (unipolar elongation), not visible during their spherical stage.

The filamentous yellow algae of the genus *Vaucheria* grow at one end only like hyphae of filamentous fungi and are thus *uni-* or *mono*polar. The auxospores of pennate diatoms grow *bi*polarly as the filamentous green algae Zygnematales (*Spirogyra,* etc.), which possess a main axis of symmetry, but with equal ends, and have been called *equi*polar or *homobi*polar. Oppositely, the green Cladophorales and aquatic filamentous fungi such as the *Allomyces,* which grow at one end by vegetative hyphae and the other by rhizoids are axially *heterobi*polar. At the organismic level of a moss, a fern or a tree, polarity also differs from one end to the other insofar as at one end of their growth axis they develop leafy shoots, whereas at the other they develop rhizoids or roots. This heterobipolarity is not merely something which concerns the overall structure of the plant body. Within a particular organ, such as root, there are polar axes in terms of developmental processes (Burgess, 1985). The apical end of these axes is the meristematic cell, the basal end is the mature cell. Sometimes there are cells with *multi*polar growth, i.e. branched systems of coenocytic fungi or algae, stellatoid-armed parenchyma cells and non-articulated laticifers; even the morphogenesis of cells with a complicated shape like the cells of the algal desmid *Micrasterias* can be regarded as resulting from multi- or *poly*polar growth.

Polarity is thus of fundamental importance to directional growth. Without stable axes of growth, plants would exist as formless masses of cells, rather in the manner of callus tissues in culture. On the other hand, if axes were irrevocably fixed from an early stage of growth, then plants could not produce the diversity of structure which in fact they do. Many important developmental changes in plants involve the creation of new axes of growth. This is most easily seen in the transition from filamentous growth to two-dimensional growth in a fern gametophyte or in the formation of a lateral root. These examples of directional growth involve an obvious and predictable change in planes of cell division and growth axes.

Cylindrical cells which grow throughout their length are in contrast to tip-growing cells such as fungal hyphae, pollen tubes or root hairs. They undergo a stress pattern, with internal turgor pressure as driving force, and their bipolar axis needs not to coincide with growth direction if the tissue tensions are strong enough to pull

the cell even in the direction of its cellulose alignment (Green, 1980). Therefore "growth direction is not always set by cell structural polarity, nor, since periclinal and transversal divisions exist, is division direction set by structural polarity. It is rather cell division orientation which determines both the growth orientation and structural polarity of the daughter cells in obligatory fashion" (Green, 1980). Control of division direction is a one-dimensional phenomenon which implies a phase of parent cell elongation followed by its separation by a cross-wall normal to the homobipolar cell axis into two cells similar in size to the original. The orientation of the division plane is set anticipated by the pre-prophase band of microtubules appearing at the line of futur contact between the prospective cross-wall and the parent side wall (Pickett-Heaps, 1974; Gunning *et al.*, 1978). This traditional "growth by cell division" is often considered as alternative to "growth by cell expansion" but "it would be better to consider cross-wall formation and cell expansion as basically separable processes and ask whether in fact an obligatory directional coupling exists" (Green, 1980).

"When designing the cell, Nature generally builts in a system of checks and balances that allow cellular activities to be closely regulated by opposing stimulatory and inhibitory influences". It is by these comments that J. L. Marx introduced his "Research Notes" (Science 30 May 1986) entitled "The Yin and Yang of Cell Growth Control" summarizing a Symposium devoted to Growth Factors (UCLA 1986). Such comments were inspired by what Ruth Sager (Harvard) called "the Yin-Yang theory of cancer" — meaning that "the loss of inhibitory responses may be just as important for unleashing the malignant potential of cells as is activation of the stimulatory forces. In fact, both may be involved...". Since, many researchers have confirmed that the uncontrolled growth of cancer cells may result from an upset balance between the positive (excessive stimulation) and negative (deficient inhibition) regulation of cell division.

Cell growth is bipolarly controlled along two inversely oriented radial axes. The "nuclearfugal" axis imposed by the structuro-functional polarity of the ER-Golgi system starting from the ER-nuclear membrane continuity to radially reach the peripheral membrane by oriented vesicular traffic has already been presented as an essential fundament of subcellular polar organization (see IV.C). The "nuclearpetal" axis initiated by external signals and conducted by biochemical canals only begins to be unravelled: the first two stages in this cell's protocol for growth and division are the initiation of the hormonal message to reproduce and the cell's reception of the message. Then the growth factor receptor conveys the signal to proteins inside the cell; somehow, the signal must reach the nucleus along a chain of proteins phosphorylated by kinases that resemble growth factor receptors. Proto-oncogenes (genes with oncogenic potential) are known to produce the receptor for several growth factors. The proteins produced by members of the proto-oncogene family are therefore kinases that resemble growth factor receptors: they lie below the cell

membrane and attach phosphate groups to tyrosine both on themselves and on other proteins. The *ras* proteins, like the *src* family of kinases, both produced by proto-oncogenes, are key links in the system for transmitting growth factor signals to the nucleus. They belong to the G-proteins which are activated by GTP and are involved in communicating hormonal messages to the interior of cells. Yet, "no one knows what *ras* proteins do once they are activated and how they might advance the growth factor signal toward the nucleus" (Pelech, 1989). Proto-oncogenes are thus involved in cell growth and division by an elaborate chemical communication network that extends from the cell surface to the nucleus "something akin to a miniature nervous system" (Pelech, 1989). When, following oncogenic action the normal flow of "nucleopetal" information is short-circuited at critical points, this would lead to the uncontrolled, apolar growth characterizing cancerous cells. About this loss of polarized growth in tumoral cells, we have already mentioned its correlation with abnormal "stress fibers" below their plasma membrane (IV.E.1).

A. MONOPOLAR

1. Outgrowth (emergence)

a) *Spherical buds*

Growth can be identified to budding when two major criteria of its accepted definition are met: de novo surface synthesis and transverse asymmetry of division. Although relatively simple and in their details unique to a few bacteria and most yeasts, these processes appear to involve the same general principle as morphogenetic processes in other types of cells and seem likely to employ similar mechanisms. They generate membrane and cytoplasmic anisotropies and involve a conspicuous polarization of secretion and growth, paralleled by isometric cell expansion of the daughter cells.

a' *Bacterial budding*

While most prokaryotes — the classical bacteria — divide by the symmetrical process of binary transverse fission, a certain number of bacteria superficially imitate the common yeasts: they reproduce by a budding process in which a larger, mature, mother cell produces, at a distinctive monopolar location, a much smaller version of itself, the bud. This daughter cell enlarges close to the size of the parent prior to its detachment from it.

Of interest is to mention that budding, non-prothecate bacteria (genera *Planctomyces* and *Pasteuria*) related to the Archebacteria lack the peptidoglycan moiety characterizing Eubacterial cell walls and possess an unidentified protein sheet instead (Stackebrandt *et al.*, 1984).

The budding process has been shown to involve de novo synthesis of surface components of the new cell — the bud — in contrast to the situation in binary fission of classical bacteria where substantial portion of each daughter cell's surface parts were formerly part of their progenitor (Sargent, 1978). That effective involvement of fresh synthesis of antigenic cell-surface components in the budding was demonstrated by Tekniepe *et al.* (1982) through the immunoferritin labelling pattern of a budding bacterium of the group *Blastocaulis-Planctomyces.* Only a very small amount of the immunoferritin label derived from the mother cell was transferred to the daughter cells where it occurred localized on one pole. This observation led these authors to comment that this localization is superficially reminiscent of the budding process in the yeast *Saccharomyces cerevisiae,* in which Chung *et al.* (1965) showed that constriction takes place partially in the old cell wall resulting in a small quantity of the old (parent) wall material being incorporated into the wall of the otherwise newly synthesized yeast bud (see VI.A.1a[2]). In aquatic *Seliberia*-like bacteria related to Pseudomonadales, it has been confirmed by immunoferritin labelling (Schmidt and Starr, 1984) that their budding occurred by processes meeting its two major criteria, namely de novo cell surface synthesis and transverse asymmetry of division.

Electron micrographs (Whittenbury and McLee, 1967) have shown in budding bacteria infoldings of the plasma membrane into an ellipsoidal, double membrane system. These authors suggested that budding has features of an asymmetric cell division which would eliminate the shearing across the complex membrane, itself required by the simple binary fission characteristic of rod-shaped bacteria. Among other unusual ultrastructural features of the budding process, Schmidt and Starr (1982) have observed "parallel-stacked" structures in both budding mother cells and large mature cells; these membranous layers are reminiscent of those, more closely packed, previously observed in nitrifying bacteria among which certain species of *Nitrobacter* which also reproduce by budding.

a[2] *Yeast budding*

The main specific processes involved in this normally monopolar process are (Pringle *et al.,* 1985): (1) the selection of a non random site of the mother cell surface at which budding will occur; (2) the formation of a ring of chitin (the "bud scar") in the largely nonchitinous cell wall at this site; (3) the localization of new cell-wall growth to the region bounded by the chitin ring, resulting in the appearance and selective growth of a bud; (4) the localization of secretion of other materials to the surface of the bud; (5) the localization of new cell-wall growth to the tip of the bud during much of the period of bud growth; (6) the balancing of tip growth against periods of uniform (isotropic) growth of the bud cell wall, resulting in the normal ellipsoidal shape of the daughter cell; (7) the migration of the nucleus from a position

within the mother cell into the neck connecting mother and bud; and (8) cytokinesis and the formation of the septal cell wall (Roberts *et al.*, 1983). Although relatively simple and, in their details, unique to yeast, these processes appear to involve the same general principles as morphogenetic processes in other types of eukaryotic cells and seem likely to employ similar mechanisms.

The site of polar budding on the mother cell of the standard yeast *Saccharomyces cerevisiae* has been variously considered to be predetermined by vectorial "bombardment" of its wall by cytoplasmic granules (Falcone and Nickerson, 1959) or endomembranar vesicles (Moor, 1967), frontal preorientation of the spindle polar body preparing first mitotic division (Byers and Goetsch, 1975), localized acidification (Turian, 1981*a*) or positioning of actin dots below the emergence site (Kilmartin and Adams, 1984).

Several lines of evidence from serial-section electron microscopic studies (Byers and Goetsch, 1974, 1975; Byers, 1981) have further suggested that the cytoplasmic microtubules were involved in the selection of the budding site and/or in directing secretory vesicles to that site and into the growing bud. In both wild-type cells and various mutants, notably the cell deficient cycle *cdc4,* bud emergence and the early stages of bud growth occurred in cells with duplicated but unseparated spindle-pole bodies. In such cells, the double spindle-pole bodies were always oriented toward the budding site, and the cytoplasmic microtubules ran from the spindle-pole body into the bud, often seeming (at least superficially) to be associated with the secretory vesicles that were accumulated there. Centrifugation of newly formed zygotes altered the typical location of the zygote first buds, but the orientation of the spindle-pole bodies and cytoplasmic microtubules toward the budding sites was retained (Byers, 1981). Involvement of these microtubules in the directed movement of secretory vesicles was also suggested by their orientation toward the site of localized alterations of the cell wall in cells undergoing zygotes formation (Byers and Goetsch, 1975). Polar growth of the bud of *Saccharomyces cerevisiae* is sustained by the directional secretory vesicle transport. It can be inhibited by tunicamycin (Vai *et al.*, 1987), an antibiotic which is inhibitory of N-glycosylated proteins involved in the G1/S transition of the yeast cell cycle.

The distribution of microtubules during the yeast cell cycle has been extensively studied by electron microscopy (Byers, 1981) and, as expected, these studies showed a role for tubulin in mitosis and possibly in bud formation. Yeast has a mitotic spindle similar in structure to those of higher eukaryotes. There are cytoplasmic and astral microtubules as well. In yeast, the nuclear envelope remains intact throughout mitosis, and functions of the centrosomes are served by spindle-pole bodies (SPBs) that are embedded in the nuclear envelope. Cytoplasmic microtubules, which grow from the cytoplasmic face of the SPB, are present during both mitosis and interphase. Yeast (*S. cerevisiae*) has one ß-tubulin gene whose disruption is lethal (Neff *et al.*, 1983).

The development of effective immunofluorescence procedures for yeast (Kilmartin and Adams, 1984; Adams and Pringle, 1984) allowed extension of these above correlations. Cytoplasmic microtubules continued to run from the nuclear envelope into the growing bud as the spindle-pole bodies separated, the spindle formed, and the nucleus migrated into the neck; moreover, it was observed that *cdc4* mutant cells often had active cell-wall growth occurring at two or more sites simultaneously. During bud growth and prior to full elongation of the spindle in the vast majority of cells, a bundle of cytoplasmic microtubules passes into the bud from the cytoplasmic face of the spindle pole body (SPB), which is positioned on the mother side of the neck region. This bundle of cytoplasmic microtubules may be involved in transport of materials into the bud as previously suggested (Byers, 1981). In addition, it would ensure that spindle elongation took place between mother and bud, rather than within the mother cell. The long cytoplasmic microtubules at either end of the spindle would also ensure a symmetric positioning of the spindle within the cell. Treatment of yeasts with the microtubule inhibitor methyl benzimidazole-2-yl-carbamate (MBC) arrested their cell division, apparently by blocking nuclear division. The arrested cells terminated development as mother cells with large buds, suggesting that bud emergence and selective growth of the bud could occur in the absence of functional cytoplasmic microtubules. However, it was not clear that the cytoplasmic microtubules were really effectively disrupted by the drug. That cytoplasmic microtubules are not required for bud growth has now been more definitely established by using cold-sensitive mutants of *S. cerevisiae* defective in ß-tubulin constructed by Botstein's group (Huffaker *et al.*, 1988). Thus, and contrarily to previous suggestions (Byers, 1981), these observations appear to strengthen the case for non-involvement of the cytoplasmic microtubules in the selective growth of the bud and the polarization of secretion. Apparently, they are *not* essential for the establishment of yeast cell polarity (Jacobs *et al.*, 1988); they are only necessary for migration of the nucleus to the bud neck, but it is unlikely that nuclear division depends on this process (studies with the *cdc24*). Botstein's model is therefore consistent with the observations that the cytoplasmic microtubules always precede the nucleus into the bud neck and the spindle-pole body is situated adjacent to the site of bud emergence. Interestingly, the so-called "shmoo" buds monopolarly emitted at their G1 phase by mating yeasts also appear to be directed by bundles of microtubules originating from the SPBs (Fig. 18B; Cross *et al.*, 1988).

Immunofluorescent staining pattern in yeast cells (*S. uvarum*) has microscopically revealed (Kilmartin, 1984) actin dots to be present as a ring around the neck of the bud. As expected, fibers are seen, but the overall pattern is dominated by the presence of the dots which almost certainly contain actin because an independent probe for actin filaments, rhodamine phalloidin, gave very similar results (Adams and Pringle, 1984; Kilmartin and Adams, 1984). Actin fibers appear to pass from the dots into the mother cell but because of the relatively large depth of focus in light microscopy,

such connectivity cannot be definitively established. As the bud continues to grow, the actin dots disappear from the neck region and transfer to the bud. With further bud growth, the dots seem to concentrate in the tip region of the bud (Fig. 18A).

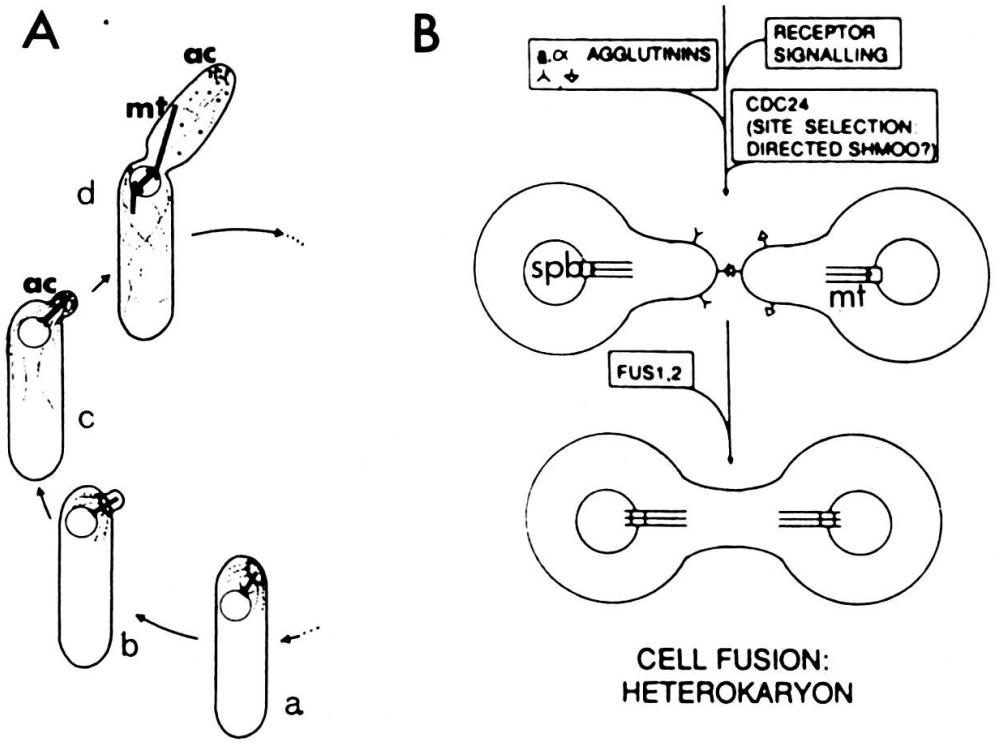


FIG. 18.

Monopolar budding of yeast.

(A) Polarized distribution of tubulin and actin in the cell cycle (stages a-d) of *Saccharomyces cerevisiae*: microtubules (mt) appear as straight lines from the spindle-pole body; actin (ac) is shown either as black dots or as fine fibers. From Kilmartin and Adams, 1984.

(B) Budding ("shmoo") of oppositely sexualized (a/α) yeast cells leading to their mating. Budding oriented by tracks of microtubules (mt) originated from spindle-pole bodies (spb). From Cross *et al.*, 1988, with permission of Annual Reviews Inc., Palo Alto, U.S.A.

In the asynchronous yeast population studied, the apparently unbudded cells could be classified as cells where budding is imminent or cells that had just completed cytokinesis. The conclusion is that, in the cells about to bud, the ring of actin dots represents the budding site, a conclusion confirmed by double labelling of fixed yeast cells with intact walls using rhodamine phalloidin and Calcofluor, a polysaccharide-specific dye that stains rings of chitin in the yeast cell wall.

Although the exact nature of the actin dots is uncompletely known (sites of anchorage of actin fibers to the membrane? actin-coated vesicles?), their distribution and that of the actin fibers strongly suggested an involvement of the actomyosin

system in localized growth of the cell wall. Specifically, during the cell cycle of wild-type cells, the dots clustered at sites of active cell-wall deposition, whereas the fibers tended to run along the long axis of the cell between mother and bud. Both the clustering of actin dots and the longitudinality of the actin fibers appeared accentuated in morphogenetic mutants that showed an exaggerated tip growth of abnormally elongated buds (Pringle *et al.*, 1986).

Investigation of mutants with morphogenetic abnormalities such as the several temperature-sensitive cellular deficient cycle strains (*cdc3*, etc.) is a promizing approach to shed some light on the mechanisms operating during the organization of the budding site. The several effects of *cdc12* mutations, i.e. inability to form a chitin ring, hyperpolarization of bud growth and inability to complete cytokinesis, have recently been viewed as secondary consequences of the loss of 10-nm actin-like filaments (Haarer and Pringle, 1987).

The accumulation of actin — a highly acidic protein — at the polar site of bud emergence might account for the sorting-out of acidic hyaloplasm detected at this elected site (Turian, 1981*a*). However, additional asymmetric protonation from mitochondria frontally-positioned along microtubules is not excluded to elect the dominant site of single budding; this mitochondriogenic acidity could thus complement the polar axiation role admittedly devolved upon the microtubular system. This interpretation would be compatible with the recently found (Matsuoka *et al.*, 1988) directional control of yeast cell budding using an electric stimulus.

b) *Cylindrical germ tubes*

Many microbial spores and plant eggs have *no* preformed developmental axis at their first, spherical stage. Pattern formation is initiated only as they acquire polarity and establish their main morphological axis (Nuccitelli, 1984).

Transcellular steady ion currents have been measured using a vibrating electrode, and found usually closely correlated with the axis of polarity (Jaffe and Nuccitelli, 1977; Harold, 1986). However, a random distribution of the proteins of ion channels/pumps over the plasma membrane, expected from membrane fluidity and lateral diffusion rates, would result in many localized current loops (for instance, Ca^+ influx — neighbouring K^+ efflux) which would provide no axis of symmetry and contribute little to the cell's overall polarity. Only by simply (but how?) separating these same channel types in space, or equivalently by separating channels and electrogenic pumps one could generate a transcellular ion flow. This pattern of ion channels "*will result* in an asymmetrical current flow through the cell which might in turn influence the cell's polarity" (Nuccitelli, 1983) and should provide a first reasonable answer to the crucial question of how a cell can recognize its front from its back!

b' *Fungal spores*

Hyphal growth starts from spore germination. Its first stage, the isometric growth ("swelling stage") of the rehydrated spore (Bartnicki-Garcia, 1973), is followed by the inception of the germ tube by determination of the presumptive site of its outgrowth (emergence). In the majority of fungi (mainly conidial Ascomycetes and Fungi imperfecti) the germ tube emerges through the spore wall at a point *not* previously differentiated from the remainder of the spore wall and the site of outgrowth is therefore undefined and unpredictable. By contrast, in most spores of sexual origin such as ascospores and, especially basidiospores, the place where the germ tube emerges from the spore is *pre*determined by some morphological characteristics of the spore (germ pore, slit, cap; see Gottlieb, 1978).

When there is no predetermined site of outgrowth, the most elusive point remains the election of that site ... Ellipsoidal or bacillus-like spores become spherical and grow isodiametrically before the emergence of germ tubes according to environmental conditions (Bartnicki-Garcia *et al.*, 1968). This initial phase of growth does not merely involve water uptake; active macromolecule biosynthesis, organelle formation and development of a new cell wall take place. Incipient buds are not morphologically different from emergent germ tubes and they soon become cylindrical germ tubes which grow apically. This pattern of germination suggests that the spores contain the information to grow isodiametrically, and it is not until certain phase of development has passed that they acquire the information for polarized growth. Polyamines have recently been implicated in that onset of polarization mechanisms and inhibition of protein or DNA biosynthesis at certain periods of the germination process can stop growth at its different steps, suggesting that specific mRNA and proteins are required at each defined growth phase (Cano and Ruiz-Herrera, 1988).

Although the mechanism underlying polarization is not understood, it seems reasonable to assume that polarization of wall synthesis is brought about by a directional change in the pattern of migration of chitosomes and other vesicles involved in wall growth (Bartnicki-Garcia, 1981). Accordingly, during the first stage

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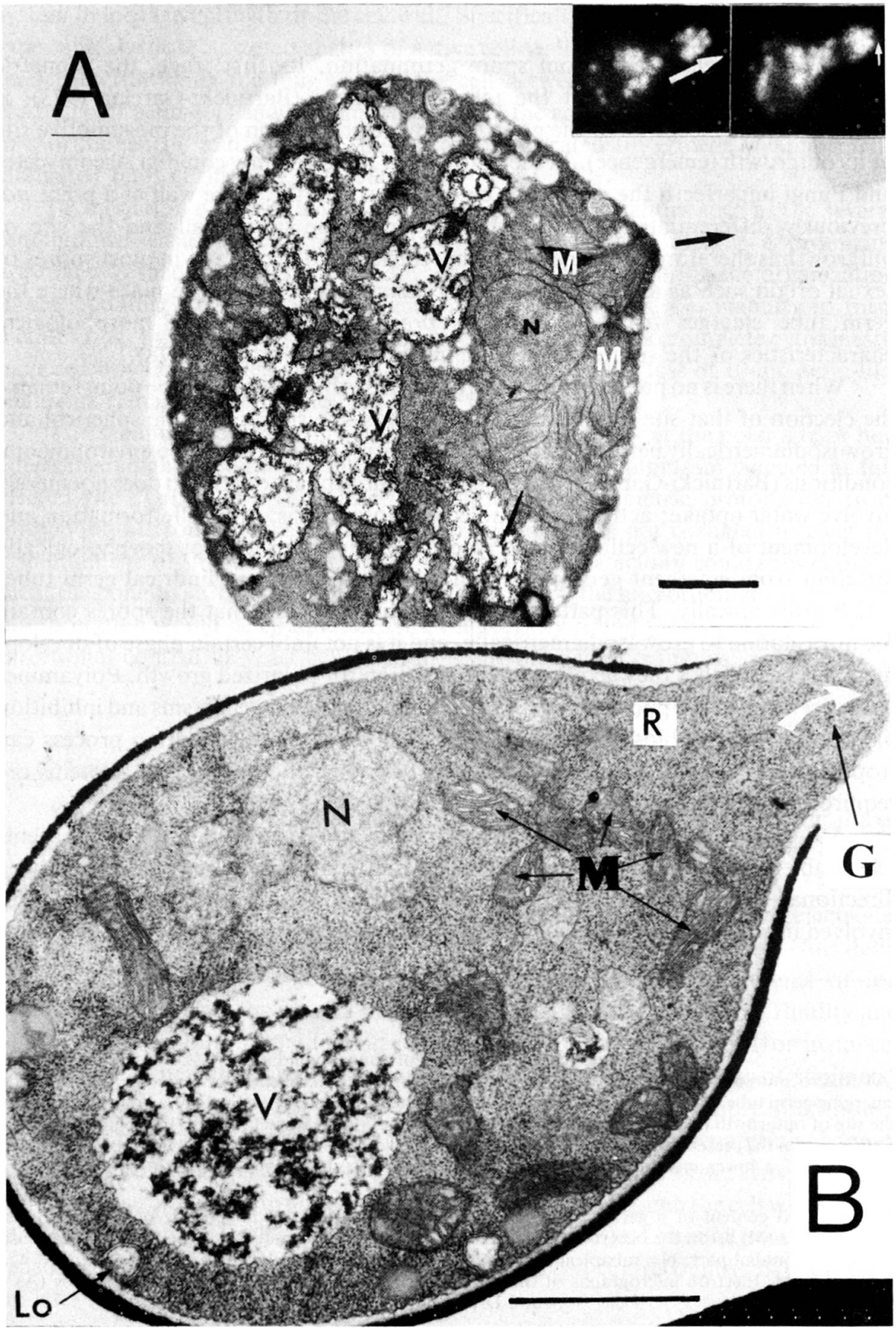
FIG. 19.

Monopolar germination of fungal conidia.

(A) Macroconidium of *Neurospora crassa* showing internal polarization of its content toward the emerging germ tube. Basally clustered vacuoles (V); subapical nucleus (N); apical mitochondria (M) below the site of outgrowth (arrow). Inserts: elongating germ tubes (large arrow) with the mitochondria vividly fluorescing in the presence of rhodamine 123: in one optical plane, a slight quenching of the fluorescence, indicative of a lower membrane potential, can be noticed in the ultimate front tip of mitochondria (small arrow).

(B) Polarized content of a germinating conidium of *Trichoderma viride* showing polarly positioned mitochondria (M) below the outgrowing (white arrow) germ tube (G) and a large vacuole (V) in the basal conidial part. N = subapical nucleus; R = endoplasmic reticulum; Lo = lomasome.

Bar = 1 μm. Electron micrographs of thin sections, obtained by courtesy of Mrs N. Oulevey (A) and Dr. J. Zuber (B).


A
V
M
N
M
V
R
N
M
G
V
B
Lo

of germination, chitosomes and other wall destined vesicles would migrate with equal frequency to any point on the cell surface. However, after a period of such spherical growth, some types of polarity signal would guide vesicles toward a target area on the cell surface where the tube would later emerge. This redirection of cell-wall synthesizing vesicles would cause the germ sphere to cease, or vastly reduce, its own expansion and produce instead an outgrowth. If polarization is maintained after emergence, a typical hyphal tube will be generated; if polarization disappears, the outgrowth will expand into spherical bud (dimorphic alternative, see A.1.c).

During spherical growth of *Mucor racemosus* sporangiospores, the levels of cyclic AMP increase 10 fold (Paznokas and Sypherd, 1975) but before germ-tube emergence, the concentrations decrease to the lower levels characteristic of hyphal cells. Cyclic AMP, added to germinating sporangiospores of *M. racemosus* during the spherical growth stage, prevented the emergence of germ tubes, and the germ spheres "swelled" indefinitely into giant cells. This correlation between cyclic AMP and germ tube emergence suggests the possible involvement of this nucleotide in the onset of polarity. The observation that fungal membrane depolarization affects the intracellular level of cyclic AMP (Trevillyan and Pall, 1979) additionally suggests a plausible link between cyclic AMP and germ tube emergence via an alteration of plasma membrane potential.

What controls the onset of polarity? There is no evidence that germ-sphere size or incubation time alone decides the time of appearance of a germ tube (germ tubes are produced from large as well as small germ spheres over a period of several hours; Bartnicki-Garcia and Lippman, 1977). The key factor(s) responsible for polarity is not known. The possible participation of structural elements, e.g. microfilaments and microtubules in vesicle migration, may provide a structural basis for directional control.

An integrated model for site election of the germ tube and its further apical growth has been proposed (Turian *et al.*, 1988) in an attempt to reconcile a) the site-determining entry of electric current loops (Harold, 1986); b) the functional polarity of frontal mitochondria (Fig. 19) positioned along microtubules (Fig. 12B) toward the outgrowing tip considered as a proton sink (Turian, 1983).

b² *Algal eggs*

The egg of *Fucus* begins its development without an inherent polarity and a differentiated axis is lacking at the time of the transformation of the homogeneous *Fucus* egg into a polarized structure can therefore be studied as a model system for the regulation of intracellular localization and the formation of biological patterns (Karp and Berrill, 1981). Polarity of the egg is expressed morphologically as a visible protrusion only at about 12 hours after fertilization and the sequence of events in *Fucus* is summarized in Fig. 20A.

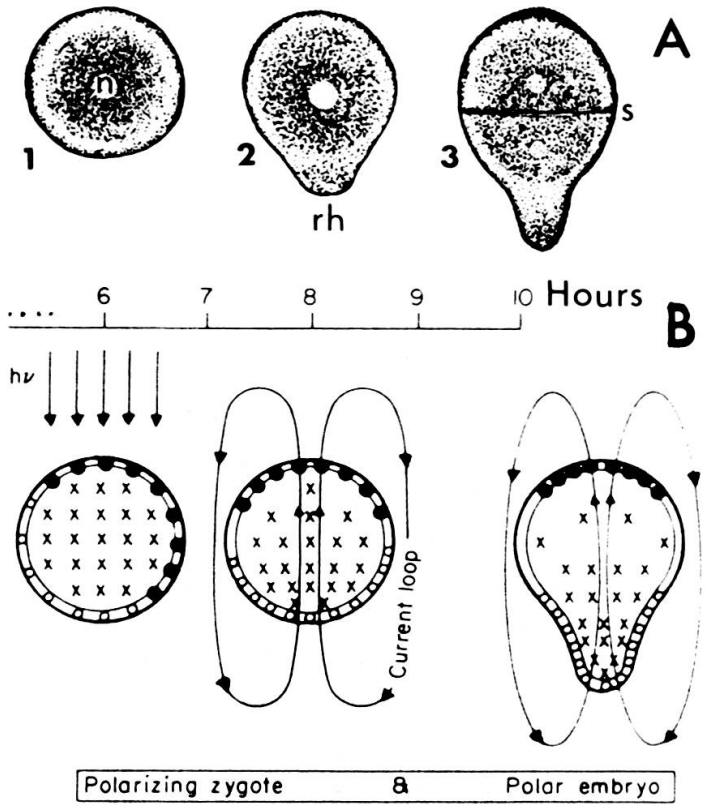


FIG. 20.

Monopolar germination of the egg of *Fucus*.
(A.1) Apolar egg: symmetrical around the central nucleus (n); (2) polar rhizoidal (rh) bump; (3) first cleavage plan with septum (s) formation at right angle to the axis of elongation. From Bonner, 1974.
(B) Light (hν)-polarization, 6 hours after fertilization, of the nearly apolar zygote of *Fucus*. The developmental process is hypothetically paralleled by activation of membrane pumps (black spheres) at the basal pole while new, ionic (K^+, Na^+, Cl^-) leaks are inserted at the outgrowing pole, the site of entrance of the electric transcellular current. Adapted from Weisenseel, 1979.

The establishment of polarity and the topographical organization of cytoplasmic components in algal eggs are accompanied if not caused by a characteristic and persistent distribution of the substances essential to axial growth. Parallely, there should occur orientation of the fine structure of the cytoplasm, i.e. of the long-chain protein molecules now known as cytoskeletal components. This was first suggested by Whitaker's (1940) studies of the effects of centrifugation on pregerminating eggs of *Fucus*: "Since the rhizoid grows out from the least illuminated side, and also from the side which is exposed to the highest concentration of diffusate from other zygotes, its downward growth towards the substrate follows naturally".

When fertilized eggs of *Fucus furcatus* are stratified by ultracentrifuging, most of the eggs remain spherical and the stratification usually persists during rhizoid formation and until the first transverse wall appears. In normal sea water, with a

pH of 7.8-8.1, in the dark, the rhizoid grows out at the centrifugal pole; but if the sea water is acidified to a pH of 6.0, the developmental response is reversed, the rhizoid being formed in the centripetal half. If the pH is between 6.0 and 8.0, the response of a population of eggs is intermediary, the rhizoids arising at random with respect to the stratification. To explain these experimental data Whitaker points out that the rhizoid typically forms at the more acid end where there is an associated accumulation of auxin, and he has suggested tentatively that the centripetal or lipoid pole (''oil cap'') has less buffer capacity than the centrifugal pole, and that its pH is therefore more affected by the pH of the medium. An internal pH gradient would result unless the pH of the medium and the protoplasm were identical, and its direction would be reversed in a medium at pH 8.0 as compared with pH 6.0. In these experiments, Whitaker also observed that ''a group effect'', i.e. the effect of diffusates from adjacent eggs, may be superimposed on the stratification effect when eggs develop in close proximity at pH 6.0 and that auxin (indole-3-acetic acid) in the medium at pH 6.3 and 8.0 tends to promote rhizoid formation at the centripetal pole. Olson and Dubuy (1937) further demonstrated that the polarity of the fertilized egg can be regulated by a local application of auxin. When an egg was placed at the end of a very fine capillary containing the growth substance in solution, the rhizoid originated towards the capillary, and the first dividing wall was at right angles to it: in the untreated controls, the rhizoid grew out at random. This and other evidence indicate that auxin is both present in the fertilized egg and is a factor determining polarity. The accumulation of auxin at some particular locus in the egg is, of course, another problem.

The orientation of the pattern emerging from the spherical cell is usually determined by internal or external asymmetries. The eggs of most species mature in an asymmetric environment and become in themselves non-homogeneous, which directs the pattern in a predictable way and no symmetry breaking is required. A rare exception is the almost homogeneous egg of the brown alga *Fucus* in which cell polarity can be directed by light, electric current (electric potential and ensuing current), differences in pH or temperature between the different sides of the egg, gradient of calcium ionophore (Robinson and McCaig, 1980), or by mutual attraction from other nearby eggs (Jaffe, 1968). This supports the view that there is an unstable situation and that any asymmetry can orient the pattern formation (Quatrano, 1978). In the absence of any orienting effect, the outgrowth appears at random but much delayed. This is also in agreement with the proposed mechanism, since the time required for the formation of an activator peak is shorter for larger deviations from the semistable equilibrium.

More experimental support to the proposed mechanism of fucoid zygote polarization by unilateral light has been provided by Weisenseel and Kicherer (1981) as summarized in Fig. 20B adapted from Weisenseel (1979).

For further polar axiation events, see VII.C.3*a*).

b^3 *Moss and fern spores*

As in many fungal spores (b^1) and zygotes of the brown algae (b^2), polarity is *not* predetermined during the ontogenesis of the spores of mosses (such as *Funaria*) or ferns (such as *Dryopteris* and *Osmonda*) and the horsetail *Equisetum*. Light is the most important external factor that polarizes these cells.

$b^{3'}$ *Mosses*

In spores of Bryophytes, polarity seems to be determined sometimes by the polarity of the spore itself in relation to its exposition in the original quartet (Wardlaw, 1952), sometimes to be induced by external differentials during germination.

Polar germination of spores *of Funaria hygrometrica* could be prevented by application of auxin (Heitz, 1940); cell division was also inhibited by this mean and "giant" cells thus produced. Von Wettstein (1953, 1965) confirmed this and found that vitamin B_1 and chloral hydrate destroyed polarity without preventing cell division. Such apolar growth continued for 50 cell generations, producing an undifferentiated, tumor-like body. Following transfer to basal medium, normal protonemal growth was resumed. Such results emphasize the importance of polar behaviour for orderly development and production of form. The point at which the rhizoids emerge from *Funaria* spores can be controlled by steady electric fields and forced calcium entry caused by the ionophore A 23187 (Chen and Jaffe, 1979). The polarization of the moss spore is thus "an autonomous process, whereas the direction of the polar axis is determined by external factors" (Schnepf, 1982).

$b^{3''}$ *Ferns*

The germination process results first in the production of a colorless rhizoid and then, at the opposite pole, in the formation of a green filamentous outgrowth. The rhizoidal germ tube was seen to emerge through a predetermined pore plugged by a polysaccharidic (?) material reddening with NaOH (Kato, 1957). As for the chlorophyllous filament, it increases in length by repeated divisions of the apical cell alone. A young outgrown gametophyte is thus a highly polar organism, with one or more rhizoids at one end of the growth axis, and a filament of cells of decreasing age at the other. This initial growth pattern is therefore primary *one*-dimensional growth (Burgess, 1985).

Apolar, very enlarged cells could be produced when spores of the horsetail *Equisetum* were exposed to colchicine (Kato, 1957). Polar germination of these spores could also be disturbed by indole acetic acid (more rhizoids) or ATP (mainly chloronema cells). In Kato's opinion, ATP tends to prevent rhizoid formation because it abolishes the physiological gradients of cell polarity. Gradients of H^+, K^+ and Ca^{2+} are also effective in inducing polarity of *Equisetum* spores (Bentrup, 1968).

b[4] *Pollen grains*

These male gametophytes of flowering plants are uninuclear cells with a standard cytoplasm enriched in storage materials; they are surrounded by a thick and highly sculptural wall containing a specialized carotenoid derivative, sporopollenin. Their content then becomes polarized and the nucleus divides to produce two daughter nuclei which are in quite different cytoplasmic environments and which have different fates. During the sorting out of the cytoplasmic components, the generative pole of the spindle structure is flattened, and preformed protein and RNA apparently move away from this position towards the vegetative pole (Burgess, 1985). The two daughter cells issued from this highly asymmetric division differ not only in shape and size but also in the nature of their organelle complement: the smallest, generative cell which is delimited by an apparently souple wall, is practically depleted in organelles and further divides into two sperm cells; the largest, vegetative cell will produce the pollen tube (see VI.A.2f).

Pollen grains drive a positively charged (K^+) transcellular ion current along their developing axis of polarity. In *Lilium grandiflorum,* this positive current (4 $\mu A/cm^2$) enters the hydrated ungerminated grain's prospective outgrowth site — the vegetative cell pole — and leaves its opposite end (Weisenseel *et al.,* 1975). Then, the current enters along most of the elongating pollen tubes and leaves around the back zone of the germinated grain.

Vesicles apparently occur throughout the pollen grain. Dictyosomes are active in the vegetative cell at late stages of pollen maturation in the anther. In mature pollen grains, they give rise to the characteristic population of vesicles (Heslop-Harrison, 1982).

c) *Spherical/cylindrical alternative = dimorphism*

As phenotypic duality in fungal cell form, dimorphism has been defined as an environmentally controlled reversible interconversion of yeast and cylindrical (mycelial) forms denoted as $Y \rightleftharpoons M$ (Romano, 1966). This phenomenon is of great relevance to medical mycology since the yeast-like phase is parasitic and temperature (37°C)-dependent while the mycelial is saprophytic. A key process in this alternative is cytokinesis since the balance between yeast and hyphal cell types may be seen, superficially at least, as some function of the relative rates of axial growth of a cell, and dimorphism as a matter of cell growth and division but intricately interwoven with morphogenesis (Stewart and Rogers, 1978).

The dividing line between a single, round or ovoid cell (= yeast) and filamentous cell (= hypha) may sometimes be difficult to discern (Scheer and Weaver, 1953). Yeast phase cells convert to hyphae by germ tube formation and polar elongation. Intermediates such as pseudohyphae (in a pseudomycelium) are yeast cells which

failed to form complete septa or to separate. A fully exclusive morphological alternative (dimorphic state) may thus not always exist.

There is compelling evidence that in *Candida albicans* the determination of form is controlled by the oxidation-reduction potential through a thiol-disulfide equilibrium (Nickerson and Falcone, 1956). Several species of normally filamentous Mucorales (grey bread molds) develop as budding yeast cells in the absence of O_2 and presence of CO_2 and the morphogenetic transition is paralleled by principally quantitative changes in the cell walls (Bartnicki-Garcia and Nickerson, 1962).

A major effector of dimorphism in *Mucor* appears to be cyclic AMP which, when added as dibutyryl cAMP to yeast-like cultures of *M. racemosus* inhibits transformation to hyphae after exposure to air. Parallely, endogenous cAMP levels in yeast-form cells decline about fourfold prior to the appearance of hyphal germ cells (Larsen and Sypherd, 1974). Among the possible roles for cAMP in the regulation of fungal dimorphism are an activation of cAMP-dependent protein kinases concerned with cell wall biosynthesis and an interaction with cytoskeleton components.

Bartnicki-Garcia (1973) proposed differential tip and wall growth as an explanation of alternative morphogeneses and has raised the issue of the origins and polarized movements of vesicles to the growing sites of cells. The flow of vesicles and cisternae to the growing point(s) of the hyphal cell wall raises the question of how do these "cargos" of wall precursors find their way to the specific growth point(s)? Cytoplasmic streaming (cyclosis), if specifically directed, could deliver vesicles rapidly to particular points in the cell. Specific mutual recognition sites on vesicles and plasma membrane or other target sites may be important in such delivery process (Stewart and Rogers, 1978). A wall which is weakened at many points may, under influence of the turgor pressure of the protoplast, expand or grow in a number of directions if deposition of new materials occurs random at weakened sites. That is, the cells would grow approximately as spheres. Likewise, apical or monopolar growth of filaments could be accounted for by deposition of new materials at weakened sites which were restricted to *one* region of the cell wall (VI.A.2). Experimental evidence suggests that for hyphae at least, weaker regions do exist at the apical tip since, when lysis is induced, it tends to occur at the tips of hyphae in *Mucor rouxii* (Bartnicki-Garcia and Lippman, 1972).

Brody (1973) asked the question of coordination of shape-determining processes at the genic levels. The topology of cell wall plasticization and microfibril synthesis should be ultimately determined by the information encoded in the cell's genome, and its expression as "morphic" proteins (Stewart and Rogers, 1983). Among the explanatory models proposed, there is that of biochemical gradients of wall effectors based on an electrochemical mechanism of movement of molecules or vesicles involving the establishment of differential ionic gradients and leading either to polarized (hyphal forms) or to generalized (yeast forms) activities. Alternatively, or complementarily, a chemomechanical system constructed by microtubules (tubulin

+ kinesin?) and microfilaments (actin + myosin) could insure the polar *or* the dispersed transport of molecules and vesicles to the sites of growth. These membranes sites might be electrically depolarized possibly in relationship with the proposed role of cAMP (Trevillyan and Pall, 1979).

The germination of a sporangiospore of *Mucor rouxii* is a clear example of the two-stage germination process common in fungal spores (see VI.A.1b[1]). In the second stage of germination, the germ sphere produces either a spherical-ellipsoidal bud or a germ tube becoming an elongated hypha depending on environmental conditions. In *Candida albicans,* pH has been uncovered by Soll (1985) as a most efficient one among the single parameters which determine the phenotype. Under a regime of pH-regulated dimorphism, he could demonstrate that the programs of protein synthesis accompanying bud and hypha formation are strikingly similar. Actin granules uniformly were found to fill outgrowing buds of *Candida albicans* while they are clustered in growing hyphal tips (Anderson and Soll, 1986). The monopolar dominance of hyphae is not only bound to actin distribution but also to ionic gradients (H^+, Ca^{2+}) which can be dissipated by ionophores with parallel hyperbranching (colonial) effect in filamentous fungi. Instead of dramatic differences in the repertoire of gene products possessed by bud- and hypha-forming cells, Soll found that subtle temporal, spatial and quantitative differences in the same architectural events appear to be basic to the genesis of alternative phenotypes. This phenotypic regulation "by no means excludes differential gene expression, but rather de-emphasizes its role" (Soll, 1987).

2. Tip growth

Most tubular cells elongating by tip growth show a polar arrangement of cell organelles along a morphological gradient spanning down from apical through subapical zones to a basal zone outgrown from the mother cell (fungal spore, pollen grain, root cell, etc.).

The *apical* zone, which is limited to the curved portion of the apex (dome), is characterized by accumulation and exocytosis of secretory Golgi vesicles. This has been shown chronologically in: root hairs (Sievers, 1963a, b; Newcomb and Bonnett, 1965), pollen tubes (Sassen, 1964; Rosen *et al.,* 1964), *Funaria* caulonema cells (Schmiedel and Schnepf, 1980; Schnepf, 1982), *Chara* rhizoids (Sievers, 1965, 1967), and fungal hyphae (Girbardt, 1969; Grove *et al.,* 1970; Roos and Turian, 1977). The *subapical* zone often has a length of many µm. In the *Chara* rhizoid, the total length of the apical and subapical zone is constant — 300 µm. The subapical zone contains active dictyosomes, Golgi vesicles, rough ER, mitochondria, and excepting fungal cells — plastids. In some cases, the apical part of the subapical zone is free of plastids.

Sometimes lipid droplets, lysosomes, and multivesicular bodies are described (Najim and Turian, 1979).

In addition to these cell organelles, the main criterion of the *basal* zone is the occurrence of the central vacuole and cytoplasmic streaming. In pollen tubes and in root hairs, cyclosis reaches to the apical body; in caulonema tip cells of *Funaria,* cytoplasmic streaming does not occur. Two populations of microfilaments occur in root hairs — bundles of microfilaments throughout the cytoplasm and single microfilaments near the plasmalemma, specifically associated with microtubules (Seagull and Heath, 1979). Both microfilaments populations are oriented parallel to the direction of and are involved in the cytoplasmic streaming.

a) *Prokaryotic microhyphae*

Polar growth is seen not only in the fungal kingdom but in Actinomycetes and related organisms such as the coryneform group of bacteria including genera *Corynebacterium* and *Arthrobacter* and Actinomycetes including genera *Mycobacterium and Streptomyces.*

Short Y-branched cells of coryneform bacteria and Mycobacteria present an unusual organization of their arm tips. In electron micrographs of thin sections of a strain of *Agrobacterium tumefaciens,* vesicular structures grouped as a Spitzenkörper-like body were even seen in the branching extremities (Fujiwara and Fukui, 1975). The growth of the narrow (average 1 μm diameter) vegetative hyphae or microhyphae of Actinomycetes like that of fungi is apparently almost entirely confined to apical regions (Gottlieb, 1953; Schuhman and Bergter, 1976). The nuclear bodies of these prokaryotes are seen dividing in these apical regions. However, in the submerged cultures of *Streptomyces streptomycini,* the active extension of microhyphae was not paralleled by an increased rate of multiplication of their nuclear bodies, a fact suggesting a possible intercalary growth, at least at some stages of culture development (Dmitrieva and Rodionova, 1971).

Time-lapse micrographies of growing *Nocardia corallina* cells have confirmed that the thin mycelium grows predominantly at the apices of transversally septate microhyphae (Brown and Clark, 1966). Subcellular structures in those apices are expected to include vesicles but more easily detectable microvesicular aggregates or "Spitzenkörper" characteristic of hyphal tips of Streptomycetous fungi have not been reported. Other differences in the apices from the rest of microhyphae include isoelectric point, increased levels of enzyme activities (alkaline phosphatase, catalase, peroxidase) as well as an increased sensitivity of apical cell wall to autolytic enzymes (references in Kalakoutskii and Agre, 1976).

All these biochemical and structural criteria provide some evidence that a functional mechanism of apical dominance functions in actinomycetous microhyphae as it does in fungal mycelia (Turian, 1969; Bartnicki-Garcia, 1973).

b) *Fungal hyphae*

Within single cells or simple organisms, polarity and axial growth can be defined in terms of direction and position of elongation within walls, or planes of cell division, or even in terms of the distribution of organelles. These aspects are most clearly seen in the growth and development of filamentous fungi. Their elongating cylindrical structures, the hyphae, are characterized by such a polarized axis extension by tip growth... "the key to hyphal growth lies at the tip" (Robertson, 1965).

The activity of the axis hyphal tip, as well as a variety of factors such as the ingredients of the medium and the temperature, affect the timing and density of branch formation in ways that can be best described as *apical dominance.* Several observations have suggested that the hyphal apex is involved in that control of branching. The decapitation experiments of Larpent (1966) first demonstrated clearly the effect of the growing hyphal tip on branch growth rate. There is a characteristic distance from the hyphal tip to the first branch for any fungus growing in culture. In the aquatic mold *Achlya bisexualis* this distance could be modified by the nitrogen source (Griffin, 1981). Maintenance of monopolar apical elongation in *Neurospora* vegetative hyphae is enforced by a high glycolytic activity in the hyphal tip sustained by a high sugar availability (Turian, 1972). This unique apical structure could therefore be considered as, at least theoretically, potentially immortal! (Robertson, 1965).

As other tip-growing cells, hyphae extend their cylindrical form by maintaining a specific gradient in the rate at which area expands within the growth zone; this rate falls, generally as a cosine or cotangent function, to zero at the base of the zone (Green, 1980). Therefore, and by contrast with diffuse extension growth along the cylindrical structure, tip growth involves localized activity at the hyphal's dome-like tip. Such hyphal growth involves the integration of cellular growth processes so as to produce an ordered sequence of events contributing to a duplication cycle which is exactly analogous to the cell cycle of uninucleate cells (Trinci, 1979). Although it is the case that the hypha is a filament composed of numerous cells connected end to end, it is essential to appreciate that hyphal growth is highly polarized, true extension growth being absolutely limited to the hyphal tip, so the whole morphology of the hypha depends on events taking place at its apex (Grove, 1978). It follows from this that the pattern of hyphae in a mycelium, which is largely a consequence of the distribution of hyphal branches, depends on the pattern of formation of the hyphal tips which initiate the branches.

As poetically described by Gooday (1983): "if you have never done so, look through a microscope at a leading hyphal tip of a fast-growing fungus such as *Neurospora crassa.* As it forges its way across your field of view, you can contemplate the rate of deposition of material at the tip, the rate of subapical synthesis necessary to sustain this, and the difficulties of biochemically dissecting this dynamic system."

Indeed, protoplasm for growth is synthesized by a considerable length of the hypha behind the tip as demonstrated by radioactive labelling (Zalokar, 1959). Structural polarity of hyphae is also expressed in cytochemical gradients: RNA, -SH groups, etc. (Zalokar, 1959); redox (Turian, 1978), pH, Ca^{2+}, HPO_4^{2-} (Turian, 1979).

The prevailing concept of hyphal tip growth is that the apical wall remains in an extensible condition by a delicate balance of wall synthesis and wall lysis with the turgor of the cell providing the driving force for wall extension (Fig. 21 A_1). Such growth criteria have been applied to ultrastructural (dictyosome-derived vesicles) and biochemical (glucans) features of hyphal morphogenesis in Oomycetes (Hohl and Hamamoto, 1977). To expand their tip area, fungal hyphae incorporate new cellular materials by dual, lytic (β-glucanase, etc.) and synthetic (glucane synthase, etc.) enzyme activities (Fèvre, 1979). Precursors for the biosynthesis of new cell wall and plasma membrane are produced in the endoplasmic reticulum all along the hypha, packaged into vesicles in Golgi equivalents, transported vectorially to the apex and exocytosed there. The anatomy and physiology of hyphae reflect their polarized mode of extension insured through intact microtubules (Howard and Aist, 1980). Among apical microtubules, a few reach the tip plasmalemma (Howard, 1981), through the basidiomycetous "Spitzenkörper" (Roberson and Fuller, 1988) or as components from a star-like structure in the *Allomyces* (Roos and Turian, 1977). The shape of the tip, the chemical composition of the wall, the distribution of cytoplasmic organelles and of cytoskeletal elements, all vary in a regular manner with distance behind the tip (Grove, 1978; Gooday, 1983; Wessels, 1986).

It seems now to be generally accepted that the materials necessary for hyphal extension growth are produced at a constant rate (related to the specific growth rate) throughout the mycelium. Under the influence of a mechanism which achieves polarized transport (Trinci, 1978a), these materials are driven towards the tip of the growing hypha. Among the materials taking part in this polarized transport are the cytoplasmic vesicles, which are thought to contain wall precursors and the enzymes needed for their insertion into the primary wall of the elongating hypha (Bartnicki-Garcia, 1973). Trinci (1974, 1978b, 1979) has argued that lateral branches are formed at locations where these vesicles (and other components) affect the rigidified wall of the hypha so as to produce a new "hyphal tip".

Polarity in hyphae manifests itself not only by the basifugal, vectorial transport of vesicles to the elongating apex but also in the electrical characteristics of transhyphal currents. The significance of electric potential in biological processes in relation to morphological polarity and polar regeneration has been recognized through the pioneer work of Elmer Lund (1947). According to Lund and his associates, bioelectric potentials in polar plants tissues are oxidation-reduction potentials developed at cell surfaces by the respiratory mechanism of the cell (Rosene and Lund, 1953). Osterhout (1922) ascribed potential difference measured to diffusion potentials resulting of the effects of salts and certain organic substances at the

inner and outer surfaces of the protoplast. Twenty-seven years ago Slayman and Slayman (1962) reported a 170 mV voltage gradient along *Neurospora crassa* hyphae with the elongating tip more positive than the trunk of the hypha. Since the membrane potential was lower at the relatively fine cellular tips where electrode penetration injury is more likely, it was difficult to know how much of the measured potential difference was natural and how much was due to a lower membrane potential measurement at the tip where the injury leak could be greater. More recently, Jaffe and Nuccitelli (1974) developed the instrumentation — an ultrasensitive extracellular electrode or vibrating probe — required to measure the minute extracellular electric fields and currents which definitely established the importance of transcellular ion currents in development.

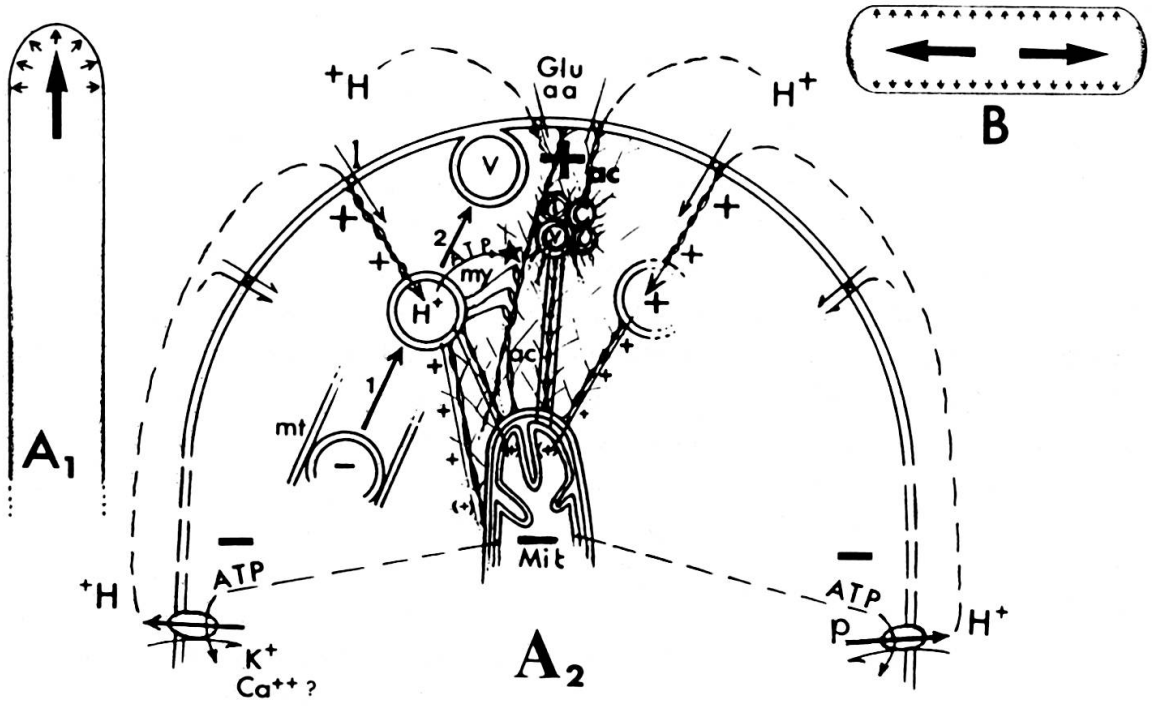


FIG. 21.

Models of polar growth.

(A_1) Fungal monopolar elongation growth from plasticized tip (small arrows) on a rigid base.

(A_2) Partially hypothetical model of proton circuitry in the hyphal tip of *Neurospora crassa:* apical mitochondria (Mit), uncoupled on their front and inside back negative, should be unable to fuel in ATP the H^+-effluxing pumps (p) of their nearby tip plasmalemma; leak-channels (1) at this level could therefore freely reintrude protons (acid tips; Turian, 1981) in symport with glucose (Glu) and/or amino acids (aa) (Harold, 1986). The presumably negatively-charged wall vesicles (V) distally generated from endomembranar elements and tracked along microtubules (mt) could act as proton sinks; the energy of their electrochemical gradient has been hypothesized to be directly transduced into local synthesis of ATP to fuel the actin (ac)-activated myosin (my) ATPase system (*) which could be the driving force of acropetally moving vesicles. Continuous provision of ATP from subapical mitochondria and thereby continuous expelling and prospective reintrusion of protons would insure self-entrainment of this "proton motor" of tip growth. After Turian *et al.*, 1985, 1988.

(B) Bacterial bipolar elongation growth from plasticized side walls (small arrows) on rigid poles. Adapted from Koch, 1985.

By the use of the vibrating probe, it has been found that many fungi drive electric currents through themselves, such that the positive charges enter the apical region and exit distally. Here, they are expelled by an electrogenic, proton-translocating ATPase and enter the apical region by symport with glucose or amino acids, particularly methionine (Harold, 1986). After its entrance into the tip, the current flows through the hyphal cytoplasm toward the distal region of outward current where protons are expelled by the electrogenic H^+-ATPase. The current loop is completed by charges flowing through the extracellular medium from trunk to tip. This proton circulation may be expected to make the cytoplasm at the tip acidic as confirmed by intracellular pH-probing (Turian, 1979, 1981, 1983) and electropositive with respect to the zone of outward current. Actin, cytochemically found to be concentrated in the tips of elongating hyphae (McKerracher and Heath, 1987), and possibly coated with hydronium chains (II.B.1), could function as electric cables in this apical proton circuitry (Fig. $21A_2$); the polarized actin microfilaments are possibly bound to a myosin-like contractile protein to drive the vesicles toward the expanding hyphal tip (Turian *et al.*, 1985, 1988).

The underlying electromechanical processes are still unclear (Jennings, 1986) and it can yet only be suggested that the electric field created by the current flow induces both cytoplasmic and membrane asymmetries by redistributing charged macromolecules and organelles (Harold, 1986; Kropf, 1986). Nevertheless, the proposal by Jaffe and Nuccitelli (1977) that currents are not only manifestations of cellular polarity, but may be causally involved in generating and maintaining this polarity has been recently questioned: doubts have been expressed about the correlation between hyphal extension and the transcellular electric current because "changes in one parameter frequently do not match changes in the others" (Takeuchi *et al.*, 1988). Indeed, the results of Harold's group would argue against the proposition that the electric field across the cytoplasm plays an obligatory role in hyphal extension. They would, however, be consistent with the hypothesis that tip proton sink and the influx of calcium ions toward a mitochondrial subapical sink (Turian *et al.*, 1985) create conditions that allow the hyphal protoplasma to extend (Harold *et al.*, 1987).

c) Algal rhizoids and filaments

The rhizoids of the higher algae, *Characeae,* are well-known objects to study factors controlling tip growth and polarity (Sievers and Schnepf, 1981). In their positive ortho-gravitropic curvature, sedimentation of the statolith complex precedes a downward bending of the rhizoid tip (Fig. 7, in Sievers and Schnepf, 1981). Interestingly, a statolith of *Chara* does not contain starch-like amyloplasts (see VIII.A.2.c[4]) but is included in a special compartment containing crystallites of $BaSO_4$. In this graviresponse, "statolith distribution and local cell wall growth marked by exocytosis of Golgi vesicles are coupled by a very simple feedback principle" (Sievers and Schnepf, 1981).

The bending mechanism of *Chara* rhizoid is a "bowing" growth, not a "bulging" growth as that of phototropically curving chloronema tips of *Dryopteris* (see VI.A.2e). The main difference between bowing and bulging concerns the behaviour of the growth center: it stays at the same point of the cell wall in bowing growth, while it is displaced in bulging growth (Hejnowicz and Sievers, 1971).

The cytoplasmic zonation of vegetative filaments of the fresh water yellow-green alga *Vaucheria* resembles that in other tip-extending cells (root-hair cells, pollen tubes, fungal hyphae). Cell elongation in the polarized vegetative filament occurs by vesicular addition in the apical zone and, for directed tip growth, nuclear cyclosis involving microtubular bands may be essential (Ott and Brown, 1974*a*). In the main apex of the green *Chara,* intracellular modifications occur during the phase preceding the segmentation of apical and subapical cells which are at the origin of a polarized distribution of the cytoplasmic and nuclear materials (Ducreux, 1979).

In *Vaucheria,* a lateral growth zone can be induced by local irradiation with blue light (max. 450 nm) of a tip-growing tube; this leads to branching within a few hours; it first results in aggregation of chloroplasts at the presumptive outgrowth site and, within a few hours leads to branching. Ionic current enters the tips of the growing tubes and leaves behind the tips. In fact, it could be concluded that an early efflux (mainly H^+ ions) and a subsequent influx (Ca^{2+} ions?) are necessary for the induction of a growth zone behind the tip of *Vaucheria* tubes (Weisenseel and Kicherer, 1981).

In *Spirogyra*, a filament grows directly out of the zygospore which has split open. In this, as in other filamentous green algae where the plantling is initially attached to the substratum, the germ shows evidence of polarity from the outset. The distal region is the locus of active growth, and there is evidence of translocation of nutrients to it. The basal or proximal region, which develops as an organ of attachment, soon becomes more or less highly vacuolated and in general appears to be a region in which the metabolism is considerably different from that of the distal region. When a zoospore of *Botrydium, Protosiphon* or *Oedogonium* germinates, it elongates and gives rise to a polarized, ovoid or filamentous structure, in which there is an evident concentration of the protoplasmic materials in the distal — apical — region.

Why the distal region should become the seat of protein synthesis is a problem about which little is known. The phenomenon is common to all classes of plants, and it may be that the same, or closely similar, factors determine the characteristic heterogeneous distribution of protoplasmic materials. Morphological features of special interest in this connection are shown by members of the green algal group of *Chaetophorales*: polarized filamentous development of the spore or zygote product; growth by an apical cell; characteristic mode of cell division leading to an incipient parenchymatization, or tissue formation. Those so-called heterotrichous developments may have a bearing on the origin of land plants.

One of the landmark discoveries in cell biology was made around 1930 when J. Hämmerling observed that the green alga *Acetabularia* is not only unicellular, but is also uninuclear during the vegetative phase of the life cycle. *Acetabularia* cells may attain enormous size (up to 200 mm in length), exhibit polarity with respect to distribution of organelles and macromolecules and, perhaps most importantly, readily survive various merotomy experiments (Hämmerling, 1936).

The life cycle of an individual *Acetabularia* cell begins with the fusion of two morphologically identical, but physiologically distinct gametes. At the moment of fusion, the structural polarity of the cell is established; the outgrowth of the apical tip of the zygote occurs towards the light and that of the rhizoid takes place closest to the nucleus (Fig. 22A). The rhizoid anchors the cell to the substrate. Since the zygote has only two chloroplasts and mitochondria, the initial rate of growth is very low.

Regeneration pioneer experiments by Hämmerling (1955) showed that, if a nucleus is introduced into a plant or plant segment which lacks one, a new rhizoid system will arise wherever the new nucleus is placed and polar behaviour of the plant may thus be modified or reversed. The anucleate fragments could be subdivided into segments that exhibited a developmental potential reflecting their original position in the stalk: the most apical fragments were capable of forming a cap, whereas the most basal fragments exhibited no growth and development (Fig. 22B). It was then assumed the existence, between the apical and basal poles, of two opposite gradients of different specifically formative, gene-produced substances. With remarkable regenerative capacities generally maintained, the original polarity pattern of *Acetabularia* resembles that of certain hydroids, but has also analogies to organs of higher plants when short pieces of the stalk develop bipolar, heteromorphic regenerates.

A transcellular electrical potential has been measured in nucleate cells and also in anucleate apical and basal stalk fragments. In fragments transferred immediately after amputation to a subdivided and illuminated vessel, a potential difference could be measured with one end becoming constantly positive and presaging the future site of regeneration (Novak and Bentrup, 1972). Therefore an electrical potential is oriented parallel to the long axis of the cell where the growing apex is positive relative to the basal region. Unfortunately, these results are still unconfirmed. It is indeed not clear how tightly-coupled morphogenesis or structural polarity in the cell is to potential differences, since application of the ionophore A 23187 suppresses cap formation, but not the potential gradient or longitudinal growth (Goodwin and Pateromichelakis, 1979).

One of the major tasks of modern biology is to reduce structural phenomena to their molecular and metabolic equivalents. This necessarily leads to the consideration that a basic part of pattern formation is the development of polarity. In this sense, polarity is closely connected to the formation of chemical gradients, as occurs

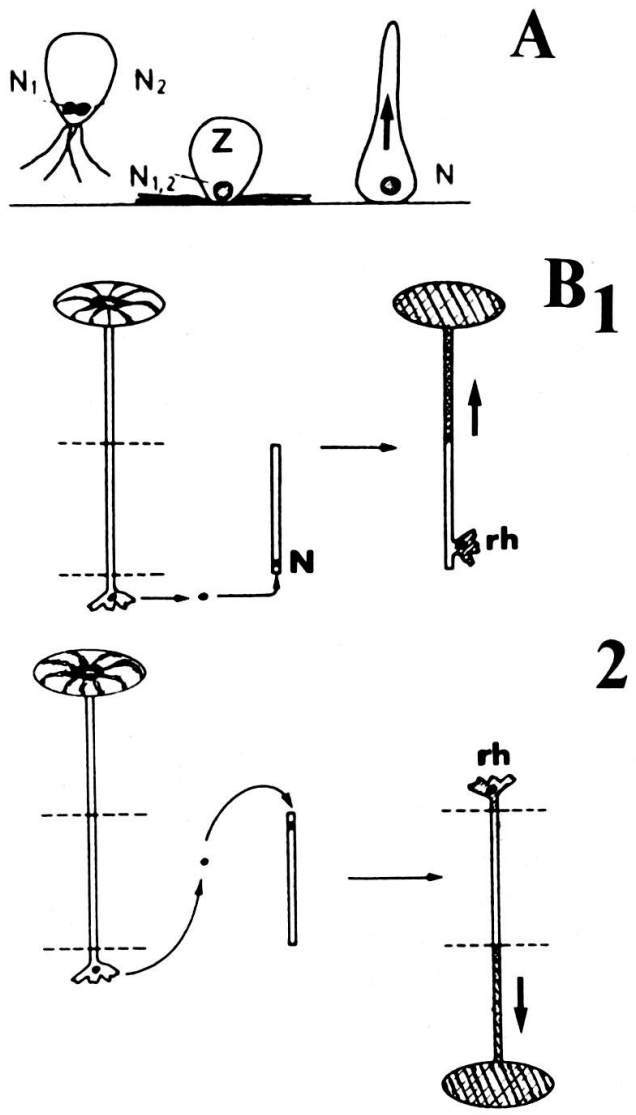


FIG. 22.
Polarity control in *Acetabularia.*

(A) Monopolar germination of the algal zygote (Z): after the tetraflagellate stage both nuclei ($N_1 + N_2$) fused in the pole; the germ tube outgrows (arrow) from this basal uninucleated (N) zone.

(B) Change of stalk polarity under the influence of the nucleus: (B_1) basal anucleate fragment cut out of a cell receives an isolated nucleus from the basal part: a rhizoid (rh) is formed in the place where the nucleus (N) is located and polar elongation and cap formation occur at the former apical part (arrow). (B_2) Basal anucleate fragment cut out of a cell receives an isolated nucleus from the basal part; the nucleated stalk fragment is grafted in an *inverse* position: a nuclear-containing rhizoid (rh) is regenerated in the place where the nucleus was located while stalk growth (arrow) and later cap formation occur at the opposite pole. Regenerated regions are striated.
Adapted from Schweiger and Berger, 1981.

in the *Acetabularia* cell, which is subjected to developmental changes that eventually give rise to a mixed chloroplast population, particularly when the stalk has reached its maximal length. The chloroplasts then show a wide morphological and physiological heterogeneity along an apical-basal gradient (Schweiger and Berger,

1981). Non regenerating anucleate cell fragments do not contain chloroplasts of the apical type. In both nucleate and anucleate regenerating fragments, the apical-basal gradient of chloroplast types is reestablished. A relationship between chloroplast heterogeneity and chloroplast-nucleo-cytosol cooperation is also indicated by the fact that the chloroplasts in the apex and in the vicinity of the nucleus look similar, presumably because they are in a similar functional state. This situation is paralleled by a high density of cytosolic 80 S ribosomes in the tip of the cell and immediately adjacent to the nucleus (Boloukhère, 1972), a site where the main part of the protein synthesis takes place. Nevertheless, the heterogeneity of the chloroplast population and the uneven distribution of the different types of chloroplasts within the cell raise the question of whether there are other subcellular structures that exhibit intracellular gradients.

The apex of the thallus of the brown alga *Sphacelaria* contains a large initial cell characterized by a strongly polarized organization. Migration of the nucleus to a distal position prior to an asymmetrical mitosis characterizes this apical mode of functioning (Ducreux, 1984).

Regenerating cells of *Griffithsia,* a marine red alga, show a polarity which can be modified by an endogenous hormone (Waaland, 1984). Rhizoidal elongation is confined to the tip of the apical cell while in a shoot filament, intercalary cells elongate in narrow bipolar bands at the top and at the bottom of the cell. Rhizoids are negatively phototropic and shoot filaments are positively phototropic. By regeneration experiments it has been shown that polarity is quite strong and does not seem to be changed by environmental factors such as light and normal gravity. Thus, in *Griffithsia* cells, "we see a striking example of positional control of development" (Waaland, 1984).

d) *Protonema (mosses)*

The relationship between the polarity expressed by growth and division and the structure of cells has been examined in polarly growing filamentous protonema of the moss *Funaria hygrometrica*: green filaments called *chloronema* arise from germinating spores; after their growth for several days as a single uniform cell type, a second type of cells is also linearly produced which is the *caulonema.*

Caulonema cells have fewer chloroplasts than chloronema, and a different pattern of proteins shown by electrophoresis. The caulonema cells can give rise to buds as side branches, and these produce the reproductive tissues at the later stage. Thus the whole plant is polar even though it is expected that overall polarity is also expressed at the level of individual cells (Burgess, 1985): caulonema cells grow from the tip, and have a rather pronounced region of cytoplasm just behind the tip which is devoid of the large cytoplasmic organelles such as the nucleus and plastids. The terminal few micrometers of the cell contain only small vesicles, and behind this a

region which contains dictyosomes and mitochondria. A region of dense cytoplasm which includes the nucleus is found next and, towards the base of the cell, is the vacuole. Thus the cell is "extremely polarized both in its growth characteristics and in the distribution of its cytoplasmic contents". All polar gradients known in *Funaria* caulonema tip cell have been well illustrated by Schnepf (1982). In this apical cell, a feedback between plasma membrane and nucleus controls polar growth, microtubules and perhaps also actin microfilaments (Schnepf, 1986).

Polar elongation of caulonema tip cells of *Funaria* is stopped by colchicine, suggesting that microtubules are effectors for polar growth — even imposing polarity (Doonan *et al.*, 1988) — based on the zonation of tip organelles. When this zonation is physically disturbed (centrifugation, etc.), regeneration of new tips occurs in front of the displaced nucleus, and it suggested to Schmiedel and Schnepf (1980) that site of growth and direction of the polar axis are determined by the position of the nucleus within the cell. This position is itself determined by the polarly organized ectoplasm (plasmalemma *plus* cytoskeletal elements). In some regeneration, the subapical cell formed a new tip which grew back towards the center of the inoculum. This type of behaviour represents a reversal of polarity of the original apical cell and confirms that the nucleus, by its position, exercises an overriding influence on the polarity of the cell in this situation (Schnepf, 1982; Burgess, 1985).

The clear dorsoventrality expressed by the division of the apical cell in leafy liverworts must be regulated by external factors. Gravity is probably an important source of positional information in the bipolar gemmae of *Marchantia polymorpha* which possess two identical apical cells on each side. Gravity determines which will divide to produce the double dorsoventral structure of the outgrowing thallus (Halbsguth, 1953).

Interesting experiments concerned with the polarity of regeneration in caulonema and sporogonia have been surveyed by Carr (1984).

e) *Prothallia (primary fern stage)*

Spore germination in many species of ferns begins with a highly asymmetric cell division. In *Onoclea sensibilis,* the nucleus moves from a central position to one end, and the spore is partitioned into two cells of unequal size: the smaller cell differentiates into a rhizoid, the larger cell and its derivatives give rise to the prothallus (Miller and Bassel, 1980).

The filaments outgrown from fern spores (VI.A.1b$^{3"}$) increase in length by repeated divisions of the apical cell alone. A young gametophyte is thus a highly polar organism, with one or more rhizoids at one end of the growth axis, and a chloronematous filament of cells of decreasing age at the other. Eventually, under appropriate conditions, the gametophyte relinquishes this style of filamentous growth by apical division and instead produces a two-dimensional sheet of cells called a

prothallus. In *Dryopteris,* this occurs by oriented divisions, at right angles to the divisions which give rise to filament extensions, followed by further divisions in the expanding prothallus (Burgess, 1985). Interestingly, this two-dimensional growth pattern could be reversed to filamentous growth when amino acid analogues were applied during the cell plate stage (Hotta and Osawa, 1958).

The plate-like organism resulting from two-dimensional growth develops first into a heart-shaped prothallium and then into a mature one that contains antheridia and archegonia, or male and female sex-cell tissues. The comparatively simple process is thus a good example of a developmental system suitable for analysis of the nature and cause of the switch from one-dimensional to two-dimensional growth. It is already known that a sharp increase in protein concentration occurs whenever two-dimensional growth takes place, and that it is associated with a change in the nucleotide composition of the dividing cells.

Along the polar axis, the division of all the cells except the apical cell is suppressed until the transition to *two*-dimensional growth occurs. The transition is dependent upon the quality of the light in which the organism is grown: in red light, filamentous, chloronemal growth continues without the formation of a prothallus; in blue or white light, the transition occurs readily; in very high intensity blue or white light, it can occur before the filament has reached the five-cell stage (Burgess, 1985).

The apical zone of the red light-grown chloronema of *Dryopteris filix-mas* shows a positively phototropic response to unilateral red light. Moreover "if the chloronema is illuminated by plane-polarized red light of equal intensity at two opposite flanks (to compensate the phototropic effects), the direction of tip growth is perpendicular to the electric vector of light" (Sievers and Schnepf, 1981). This bending response is called *polarotropism.* Blue light receptors and phytochrome are involved in the polarotropism of *Dryopteris* tip cells, and the photoreceptor dipoles are oriented parallel or perpendicular to the plasmalemma of the apical zone (Etzold, 1965; Steiner, 1967).

f) *Pollen tubes*

Like hyphae, actively growing pollen tubes show tip zonation: organelles, mitochondria especially, are excluded from the clear apical zone of the tube (Heslop-Harrison, 1979). Such a topological situation which mimics the exclusion zone of hyphal tips makes the wider pollen tube an especially favourable material for cytochemical-physical studies concerning polarity phenomena: the cytoskeletal elements of pollen tube can be demonstrated with fluorescent probes for tubulin and F-actin (Lloyd, 1987). Recently, a dense three-dimensional net-axial distribution of actin filaments was detected, by rhodamine-phalloidin staining, along the length of permeabilized pollen tubes, including their extreme tip (Pierson, 1988).

In *Lilium* pollen tube there is a distinct zonation of organelles at the tip. Numerous secretory vesicles, believed to be derived from dictyosomes, are concentrated at the apex of the tube, the region of cell wall formation (Van der Woude *et al.*, 1971). They originate behind the subapical front of the mitochondria, namely from the Golgi elements. Their basifugal movement to the apical membrane has variously been ascribed to cytoplasmic streaming implicating microfilament sliding (Franke *et al.*, 1972; Pierson *et al.* 1986) or to self-electrophoresis (Jaffe and Nuccitelli, 1977). This last hypothesis is founded on the electric positiveness of the tube apices contrasting with the negative charge of the polysaccharide wall precursors in the vesicles. This positiveness was experimentally shown by the fact that a positive current, mainly carried by K^+ ions, enters the elongating zone and leaves around the back zone of the tube (Weisenseel *et al.*, 1975). The ionic gradient could indeed provide the electrochemical force to drive the vesicles to the advancing tips. Moreover, the selective entrance of K^+ at the tips would occur in exchange of H^+ ions extruded from inside the tube (Weisenseel, 1979). These endogenous protons could be responsible for the differential acidity detected in the thereby gelified apices and sides of elongating pollen tubes (Turian, 1981*b*).

Elongating tubes also show a gradient of total Ca^{2+}, with Ca^{2+} higher in the apical region according to both low temperature autoradiography (Jaffe *et al.*, 1975) and chlortetracycline fluorescence (Reiss and Herth, 1978). As for the Ca^{2+} influx channels, they would be segregated by the plasma membrane which thus appears to control the establishment of the cell's axis of polarity. However, tip extension and vesicles fusion were shown, with Ca^{2+} antagonists, to be independently Ca^{2+}-controlled processes (Picton and Steer, 1985).

A case of magnetotropic response has been reported in pollen tubes of *Lilium longiflorum* (Sperber *et al.*, 1981). If pollen grains germinate and their tubes grow in the homogeneous magnetic field (14 Tesla) of a horizontal Bitter magnet for 3 hours, the tubes are oriented parallel to the magnetic field with equal tendency to grow toward the north or toward the south pole. This magnetotropic response becomes weaker with decreasing field strengths. In unhomogeneous fields, the pollen tubes grow preferentially toward the region of decreasing field strength. The authors speculate that the magnetic fields may act on the plasmalemma where they might influence the localization of membrane proteins necessary for exocytosis of vesicles.

g) *Root hairs*

When epidermal cells mature, some of them may form an elongated extension or root hair, functionally devoted to the absorption of soil water and nutrients. Inception of the hair occurs at the level of a localized disposition of polysaccharides at the outer epidermal cell wall (Clarke *et al.*, 1979). In many species of plants this site is towards the apical end of the cell and indicates a polarity within the epidermal

cell at the time when it is competent to form a hair. Microtubules are arranged just beneath the plasmalemma in close relationship with the cellulose microfibrils of the developing cell wall (Newcomb and Bonnett, 1965).

Rapid growth of the hair occurs by the incorporation of dictyosome-derived wall material at the tip. The polarized deposition of this material may be related to the polarized flow of protons through the hair — protons entering at the growing tip and leaving at the base (Weisenseel *et al.,* 1979). In an elongating root hair, microtubules are present in the cortical arrays, except at the tip of the cell which, as in fungal hypha, is the site of polar growth.

Experimental evidence suggests that all epidermal cells have the potentiality to form hairs. However, none appears under the root cap and any division that occurs in the polarized epidermal cell causes the hair-forming potential to be confined to one of the two daughter cells (Filipenko, 1980, cited by Barlow, 1984). The site of hair emergence could not be displaced by centrifugation; this has suggested to Nakazawa and Yamazaki (1982) that it is controlled by a stable zone of cortical cytoplasm. At the molecular level, this positional control might involve a cytoskeletal component such as microtubules (Gunning, 1982). In an elongating root hair, microtubules are known to be present in the cortical arrays, except at the tip of the cell which, as in fungal hypha, is the site of polar growth (Barlow, 1984).

As in other tip-growing structures, electric fields have been detected in outgrowing root hairs. It has been suggested that protons flow into the apical end of the cell and here help to initiate growth of the hair (Weisenseel *et al.,* 1979).

h) *Insect bristles*

The bristle of the bug *Rhodnius* always grows out in a posterior direction. It is therefore evidently polarized: if an area of the epiderm is killed by burning, it is restored by the inward migration of the surrounding epidermal cells. At the second moult after this wound-healing process, new bristles are differentiated at the characteristic intervals; the hairs grow backwards, except in the central zone where the converging cells have met. Thus, the epidermal cells might have an inborn polarity which was retained as they migrated inwards across the wound (Wigglesworth, 1966).

Polarity gradients also appear to intervene in the development of bristles of the other bug *Oncopeltus* (Locke, 1960) in which the cells are responding to a gradient of some chemical component present in the epidermis which would show a graded concentration from the anterior to the posterior boundary of each segment.

i) *Animal neurites*

Despite the great diversity of shapes exhibited by different classes of nerve cells, nearly all neurons share the double feature of: the *growth cone* by means of which

two types of developing processes or neurites (in tissue culture), the axon and several dendrites, advance toward their destination, and the *synapse,* which the process forms when it arrives there (target innervation). The two types of processes differ in morphology, rate of growth, macromolecular composition of their cytoskeletons and surface membranes, as well as synaptic polarity.

For nerve cells to develop their highly polarized form, appropriate structural molecules must be targeted to either axons or dendrites. This process could be achieved by the synthesis of structural proteins in the cell body and their sorting to either axons or dendrites by specific transport mechanisms. Messenger RNA for a cytoskeletal protein (MAP2) has been selectively localized in dendrites (Garner *et al.,* 1988). The expression of a neuron specific, membrane-associated phosphoprotein (GAP-43) is sharply elevated during neuronal development and regeneration (Benowitz and Routtenberg, 1987). Quite recently, Goslin *et al.* (1988) have shown that this GAP-43 is compartimentalized in developing nerve cells and have thus provided the first direct evidence of important molecular differences between axonal and dendritic growth cones.

It was already known that the two types of processes differ in their morphology, in their rate of growth, in the macromolecular composition of their cytoskeleton and surface membranes, and their synaptic polarity. As noted by Dotti and Banker (1987), transsected axons of mature neurons keep their fundamental polarization, develop quite normally and the cell can still establish a single axon and several dendrites.

Although in most types of neurons the axon terminals contain enzymes for neurotransmitter synthesis and perform a great deal of local recycling of synaptic vesicle membrane, there is a continuing need for supplies of freshly synthesized membranes and enzymes from the cell body. The neuron can thus be viewed schematically as a secretory cell in which the site of exocytosis — the axon terminal — lies at a great distance from the site — the cell body — where the macromolecules and the secretory vesicles are first formed. This mode of organization creates a need for a rapid axonal transport mechanism.

The neuronal cytoskeleton consists chiefly of neurofilaments, microfilaments (actin) and microtubules (tubulin). It maintains the elongated structure of the neuron and provides for transport of materials to and from the cell body, where proteins and lipids are synthesized for use elsewhere in the cell. The vesicular traffic along the axon, conveying lipids, membrane glycoproteins, and materials for secretion, constitutes the fast component of axonal transport. In addition, there are slow components of axonal transport, whereby the proteins of the cytoskeleton are themselves steadily exported from the cell body, together with enzymes of the cytosol. Membranous vesicles of various shapes and sizes can be detected in transit along the axon (Fig. 23). Axonal transport has fast *antero*grade (outward) and *retro*grade (inward) transport components, consisting of vesicles moving at speeds of up to 400 mm per day, and slow anterograde components, carrying proteins of the

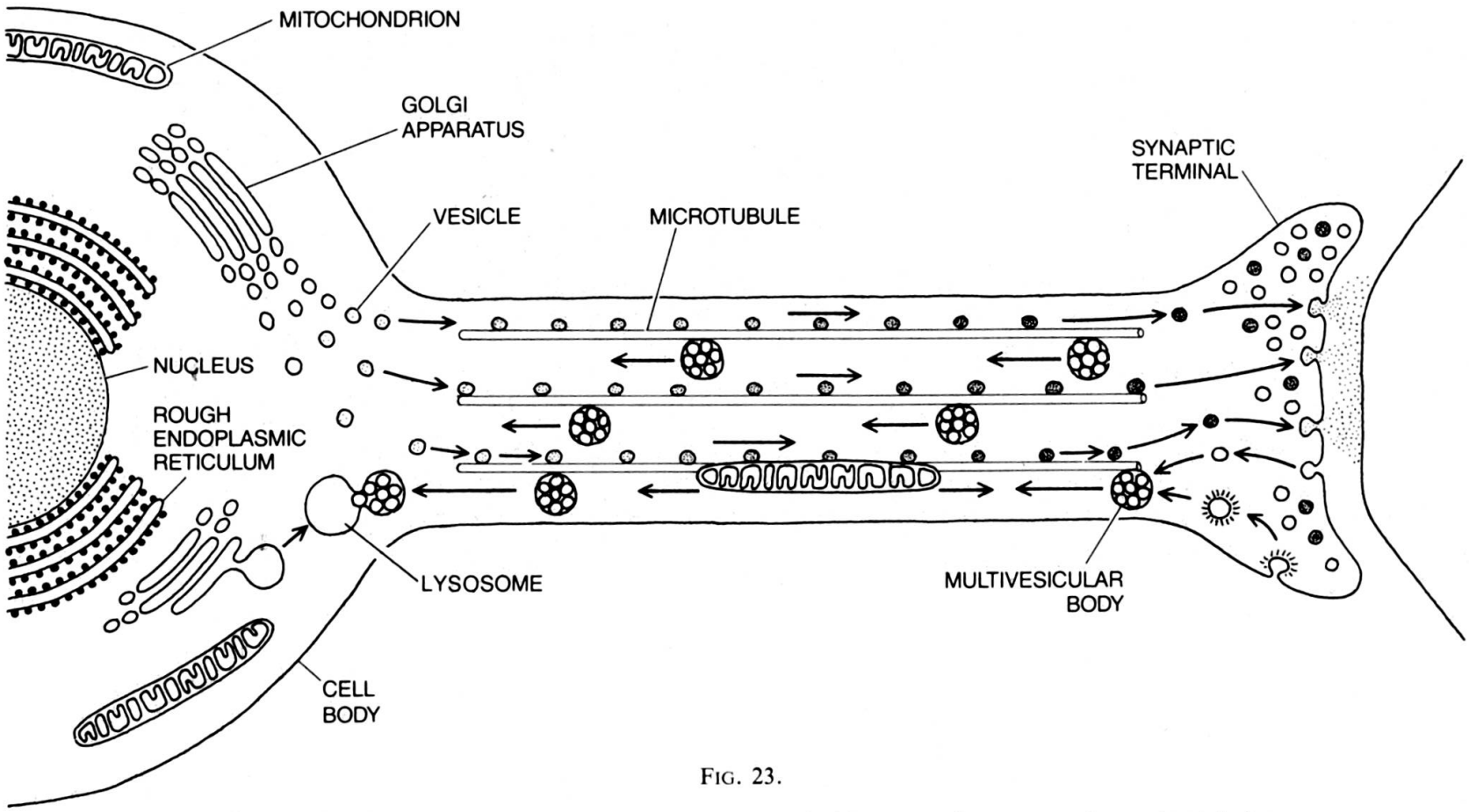


FIG. 23.

Schematic view of neuronal — axonal and synaptic — polarities. Axonal transport along microtubules allows the swift exchange of substances between the nerve cell body and the synaptic terminal at which the nerve fiber adjoins its target cell. Vesicles that will convey neurotransmitters are manufactured by the Golgi apparatus and carried toward the synapse. Surplus membrane at the synaptic terminal is packaged into multivesicular bodies, which return to lysosomes in the cell body for degradation. Arrows pointing from left to right illustrate anterograde movement of vesicles and their polar transport across the synaptic terminal; arrows pointing from right to left illustrate retrograde movement of multivesicular bodies and lysosomes. Mitochondria move in both directions, catering to the energy demands of the cell. Transport of the vesicles is continuous while that of the mitochondria is intermittent. From R. D. Allen, Scientific American 256: 26-33 (1987), with authorization.

cytoskeleton and cytosol at speed of a few mm per day (Alberts *et al.*, 1983). In their *bi*directional saltatory motion, membranous vesicles can move in either direction, despite the fact that microtubules have a distinct structural polarity. Therefore, the motor cannot be accomodated by models as those of myosin crossbridges for muscle contraction or of the dynein sidearms for ciliary beating. A three-component system of transport reconstituted from squid axoplasm has been proposed (Vale *et al.*, 1985*b*): vesicles — isolated microtubules — soluble mechanochemical proteins. In this system, vesicle bidirectional motility is ATP-dependent and the directionality of the force-transducing motor which is independent of the monopolar track-microtubules, have recently been shown to be dually insured by cytoplasmic dynein for *retro*grade transport and kinesin (Vale, 1987) for *antero*grade movement of vesicles. These contractile proteins thus provide motive forces for inverse monopolarities.

In neuronal cytomechanics, it should be important to distinguish between motility involved in neurite guidance and the process of neurite elongation itself (Bentley and Toroian-Raymond, 1986). What part, then, does the *growth cone* (Cajal's term) play in the polar assembly of the materials required for neurite elongation growth? This specialized structure consists of a bulbous expansion of the axon tube from which lamellipodia and a number of filopodia or microspikes protrude in all directions. These thin processes are distinct from axons and growth cones in that they do not contain mitochondria, neurofilaments or microtubules but, rather, contain a prominant network of actin microfilaments. The filopodia continually extend and retract, appearing to receive orienting signals for the directional growth of the axon. The growth cone and its filopodial expansions is thus primarily an organ of locomotion which must adhere to the substratum over which it advances, and this advance depends on the actin filaments. If cytochalasin B is added to the culture medium, to prevent the polymerization of actin into filaments, the growth cone halts its microspike activity and locomotion. Nevertheless it continues to adhere to the substratum, and the neurite maintains its length. By contrast if colchicine, which disrupts microtubules, is added to the culture medium, the neurite retracts toward the cell body. At the same time, by some types of changed polarity, new microspikes and even new growth cones develop from the proximal regions of the neurite. The role for microtubules is therefore to serve to stabilize the elongating neurite and to restrict the sites where growth cone activity can occur. In short, "without microtubules, the developing neurite retracts; without actin filaments, it cannot advance" (Alberts *et al.*, 1983). The locomotory motility of growth cones appears to result from two cyclic and metabolically driven cytochemical processes: the ATP-energized actin polymerization cycle and actin-myosin interaction. While the involvement of actin in motility is on fairly solid ground, the evidence for myosin involvement is still circumstantial (Smith, 1988).

The broad "palm" of the growth cone is full of small, irregular anastomosing membranous vesicles, rather like smooth endoplasmic reticulum. The origin and possible function of membrane stacks, and the polarized arrangement of organelles have been explored by Cheng and Reese (1987) in quick-frozen optic nerves of chick embryos. They proposed that the membrane stacks are a pool of internal membranes waiting to be added to the plasmalemma of the growth cone. Polarized membrane cycling is a known feature of motile cells (see V.B.5) where it may be driven by the flow of actin-containing cytoskeletal structures in the underlying membrane cortex (Bray and White, 1988). In the growth cone, where actin-rich filopodia and lamellipodia form at the apex and sweep laterally, the actin flow follows a path similar to that proposed for membrane stacks. Thus, "directional movement of the actin cortex could be coupled to polarized membrane flow between sites of exocytosis at the tip and endocytosis at the base of the growth cone" (Bray and Hollenbeck, 1988).

Polar elongation of the growth cone stops when it arrives on its target, synapses must be formed, and outgrowth must cease. Action potentials might halt this growth cone elongation so that nerve cells can begin to communicate with each other. Creation of a synaptic terminal requires a switch in the operation of the molecular machinery at the end of the neurite so that the microtubules and neurofilaments terminating there are disassembled or degraded as fast as they arrive. This switch in the behaviour of the cytoskeleton at the nerve terminal must be accompanied by a change in membrane turnover also. In the case of a developing dendrite that forms a postsynaptic specialization, exocytosis and endocytosis must largely cease, while in a developing axon that forms a presynaptic terminal, the perpetual rapid exocytosis and endocytosis of the growth cone must give way to the Ca^{2+}-triggered exocytosis and subsequent endocytotic membrane retrieval that underlie synaptic transmission (see Darnell *et al.*, 1986).

The growth cone of certain types of neutrons must not only recognize their target but also be guided to it. Motility involved in neurite guidance and the process of neurite elongation itself may, however, be distinguishable on the molecular level (Bentley and Toroian-Raymond, 1986): guidance at the growth cone may be mainly the realm of the mechanochemical protein actin, while elongation is more fundamentally dependent on microtubules and their tubulin subunits (Smith, 1988). Because myosin is present in growth cone lamellae (see above), an actin-myosin interaction would explain the observed retrograde actin translocation (Fig. 13).

Mechanisms of directional growth of axons may include mechanical or chemical guidance in ionic channels, guidance by spatial gradients of positional markers, gradients of temporal (maturation) markers or specific inter-axon interactions (ref. in Bonhoeffer and Huf, 1985). Growing axons can also be guided by other cues (Blair *et al.*, 1985) such as adhesive substrates, diffusible factors, electrical fields. In nerve galvanotropism, there is enhanced growth toward the cathode and reduced growth toward the anode in small steady fields in a variety of neurons: chick dorsal root

ganglia and frog neural tube (Robinson and McCaig, 1980; Nuccitelli, 1984) and goldfish retina (Freeman *et al.*, 1981).

Polarity of intrisinc information might also guide outgrowth (Solomon, 1979). Growing processes must find those regions of neuropile where their targets reside and cell-cell interactions must intervene through chemotactic molecules. The nerve growth factor (NGF) which has been identified and purified in the 1950s by Rita Levi-Montalcini and Stanley Cohen is known to be synthesized in the target region and acts via specific membrane-bound receptors located on the neuron. NGF has been further characterized as a peptide hormone which regulates gene expression by several distinct mechanisms among which protein phosphorylation and ion flux (Banker and Waxman, 1987).

Axons select pathways by recognizing specific cues in their environment. These cues include cell surface and extracellular matrix molecules that mediate cell and substrate adhesion and axon fasciculation, molecules with contact-dependent inhibitory properties, and diffusible tropic factors. Even though important the insight already obtained into the strategies used in axonal pathfinding, it has not yet provided a complete description of the guidance mechanisms that operate for a single vertebrate neuron from the time of its differentiation to the establishment of its synaptic connections (Dodd and Jessell, 1988).

B. BIPOLAR GROWTH (EQUIPOLAR, HOMOPOLAR)

In the asymmetric growth model of rod-shape bacteria, cell extension first occurs from one pole only. After septation is initiated, the old growth zone continues polar length extension while a new one starts at the other pole. Thus, the growth process preceding bacterial scission can be considered as temporarily bipolar. There is also a lack of synchronism in the sequential outgrowth of the two germ tubes emerging from the opposite poles of certain species of fungal conidia.

In the bacterial and yeast-fungal cases, both poles appear to be morphologically and functionally equivalent and we will further describe their outgrowing processes as *equipolar* or *homobipolar*. By contrast, when the first outgrown germ tube is that of a rhizoid and, later, the second tube emerging at the opposite pole of the spore egg is an hypha (*Allomyces*) or a green thallic cell (Fucoid algae, Cryptogams), then can we speak of *heterobipolar* growth. However, the oppositely growing structures are not comparable, heterobipolarity implying a process of differentiation superposed on that of the primary process of bipolar axiation. This heteropolar disjunction of both form and function thus appears to be more complex, especially in green organisms which exhibit a sharper alternative colorless basal pole — green thallic pole resulting from an unequal division. Therefore, it will be considered as a process of bipolar differentiation (VII.C.2-3).

a) *Bacterial elongation*

Most prokaryotes divide by binary transverse fission, a process in which the cell elongates bipolarly along its longitudinal axis, contructs a central transverse septum and separates into essentially identical daughter cells approximately equal in size; symmetry is maintained with respect to both longitudinal and transverse axes.

Bacteria grow by enlarging their envelope in such a way that osmotic pressure does not normally cause physical rupture. The strategy of *Bacillus subtilis* for both cylindrical elongation and pole formation is now substantially defined: side-wall growth takes place by laying down new peptidoglycan, which is then displaced outwards, stretched and discarded; cross walls are laid down in the absence of stress, and then stretched and bulged outward as the septum is split and the pole is formed.

The pattern of extension growth of *Escherichia coli* has also been studied with inducible phage receptors found in the outer membrane. On transferring the bacteria from a permissive to a restrictive temperature, the phage receptors were retained in the equatorial region, while new surface without phage receptors was formed at the poles. Begg and Donachie (1977) could thus argue that the unlabelled area represents true surface extension, as there is no change in the location of the existing phage receptors. When cells of *E. coli* reach a certain critical length, which is constant in all growth conditions and equal to twice the minimum cell length, they abruptly increase their rate of elongation and divide about 20 min later. Chromosome replication terminates at about this same cell length but is not the signal for the change in rate of cell elongation. Indirect marker methods have permitted to measure the direction of elongation of living bacterial cells: when this elongation of cells is followed on minimal medium (generation time 60 min), growth is almost invariably *uni*directional, by elongation of one end only — the one which was formed at the previous division. When cells are grown in rich, broth medium (generation time 24-30 min), single cells grow by elongation in *both* directions relative to the agar surface. Both sets of observations thus indicate that growth is asymmetrical and *uni*directional in cells which have less than a certain critical length while, in longer cells, growth is symmetrical and thus *bi*directional (Fig. 20B).

To provide a conceptual framework for these new observations, Koch (1985) has developed a simple model of cell growth in which he referred to a cell of a minimum length of about 1.7 μm as a "unit cell" which can therefore be defined as the smallest cell of a given strain that can exist in any growth conditions. A unit cell has a single membrane growth site located at the pole of the cell which was formed at the previous division; net synthesis of new membrane takes place asymmetrically at the growth site, so that during growth this site remains at a fixed distance (1.7 μm) from the older of the two poles of the cell. Consequently, when the cell has grown to twice its initial length (that is, to two unit cell lengths) the growth site is in the center of the cell. If the cell divides at this length (as it will do if the cell is in minimal

medium) the division furrow will pass through the position of the growth site. Each of the two daughter cells is then assumed to receive a growth site at this newly formed pole and therefore to commence growth at this pole. Because such sister cells are now observed to grow in opposite directions from one another, it is assumed that the two newly formed growth sites have opposite polarities to each other. This model of the "unit cell growth" can be seen as a logical extension of the model first proposed in 1963 by Jacob *et al.*, which suggested a mechanism by which the spatial segregation of copies of the bacterial chromosome could be coordinated with cell growth.

Burdett and Koch (1984) have recently suggested a complementary model for pole formation, the "split-and-stretch" model based on the stability of pole wall relative to side wall and the shapes of nascent pole (electron microscopy). The key assumption of this model has been supported experimentally by the finding that "normal pole formation occurs from completed crosswall of autolysin deficient mutants in the presence of lysozyme when further murein synthesis is blocked with vancomycin" (Koch, 1985, 1988). Polar caps material (anionic polymer of the techoic type) is thus better conserved than that of the cylindrical wall region (Clarke-Sturman *et al.*, 1989).

b) *Yeast elongation*

The fission yeast *Schizosaccharomyces pombe* shows double highly polarized growth (Mitchison, 1970; Johnson *et al.*, 1982). The cylindrical cell of *S. pombe* grows exclusively but equally at the poles without detectable change in the cell radius; during the first half of the cell cycle, cells grow *uni*polarly at the primary end, which is located opposite the pole of the previous division, and during the second half of the cell cycle, the cells grow *bi*polarly (Streiblová, 1981). In the growing pole(s), numerous vesicles filled with cell wall synthetic enzymes and precursors accumulate via actin-mediated transport (Marks and Hyams, 1985).

Rules have been defined in the patterns of extension growth in the fission yeast *Schizosaccharomyces pombe.* Thus sibs with old walls which grow monopolarly at the new rather than the old end violate Mitchison's rule (1970) while both sibs whose progenitor grew at both ends, and are therefore bipolar, always grow at the old end and follow Mitchison's rule (Miyata *et al.*, 1986). By quantitative autoradiographic analyses approximately 20% of cells were found to be bipolar by incorporating glucose at both ends (Johnson, 1965) while by fluorescence microscopy 80% of the cells were bipolar (Streiblová and Wolf, 1972). Among the spheroidal fission yeast cells resulting from treatment with the antifungal antibiotic aculeacin, cells were found whose putative growth axis and polarity differed (orientation of the septum) from those of their progenitor (Miyata *et al.*, 1985). The problem of polarity of cell wall growth during recovery from antibiotic treatment, the nature of the internal

changes occurring during these morphological alterations, and the coordinate regulation of α and ß-glucans during normal and aculeacin-disturbed yeast growth are not yet understood.

c) *Yeast double budding*

Bipolar budding is characteristic of the apiculate yeasts, e.g., the genera of *Nadsonioideae* (Hayashibe, 1975). In this form of budding, permanent bud scars are also left on the cell wall. Most ellipsoidal strains of yeasts bud mainly from both poles so far as the 3rd and the 4th buddings are concerned; the budding positions are located at the loci with the maximum curvature. Nickerson (1963) first suggested that buds arise through permitted loci of the maximum curvature of the yeast cells. However, it is to be noticed that the positions and sequence differ according to strains and the culture conditions. The finding that rather irregular budding occurs in spheroidal cells will be explained by their uniform curvature of the cell wall.

Bipolar budding occurs in *Saccharomycodes ludwigii*: successive generations of buds arise at the same site on the cell wall resulting in a series of concentric bud scars (Gay and Martin, 1971).

d) *Fungal double germ tubes*

Genetically determined, a majority of conidia of the brown-rot imperfect fungi of the form-genus *Monilia* germinate by semi-synchronous outgrowth of two germ tubes, one at each pole with maximal curvature of their ovoid conidial cells. It has been proposed that the enlarging central vacuole may play a role in the bipolar segregation of two lots of mitochondria electing opposite sites of germ tubes outgrowths (Turian, 1985*a*).

Bipolar germination is the normal pattern occurring in the genus appropriately named *Bipolaris* (see Alcorn, 1988).

e) *Algal cells elongation*

In pennate diatoms such as *Cymbella,* the zygotes issued from conjugation of gametic cells soon elongate bipolarly to form auxospores. These diploid spores then secrete a bivalval frustule composed of an amorphous silica gel ($SiO_2.nH_2O$) enveloped by an organic layer. After cytokinesis, deposition of the new frustular valve is a "directed" growth that starts at a "primary silicification site" in the center of the new cell surface (raphae area) and gradually proceeds to the margins. In the developing valve of *Navicula,* this site was observed by electron microscopy to correspond with a "silica deposition vesicle" which extends polarly the length of the cell along the apical axis and beneath its plasmalemma (Schmid *et al.,* 1981). In

diatoms, regulatory processes such as signal inhibition of over-elongation appear to be still largely ignored.

In the green alga *Nitella,* elongation of the long cell which occurs throughout its length is based on a physical anisotropy of the wall. The transverse arrangement of strong cellulose microfibrils renders the wall strong in the transverse direction, so that it grows primarily in length. The orientation of the synthesis of the fibrils can be shown to be independent of the arrangement of the pre-existing fibrils and thus oriented synthesis is based on order in synthetic machinery or a "cytoplasmic framework" (Green, 1980).

If growth depends upon the yielding of the wall to the vacuole pressure, then any directed features of cell expansion must have a basis in the properties of the cell wall. Hydrostatic pressure is the same in all directions and directed growth has to be controlled by the unequalness of yield (physical anisotropy) of the wall. Such physical properties have been found for the slow yield of the *Nitella* internode cell (Probine and Preston, 1962) in which the wall yielded relatively easy in the major, longitudinal direction of growth and very strong in the transverse direction.

Intercalary shoot cells of the red alga *Griffithsia pacifica* elongate by a unique process termed *bipolar band growth,* which involves wall extension being localized to two narrow bands at each end of the cell. Elongation of internode cells of *Nitella axiliaris* is also connected with a banding pattern, growth being almost entirely restricted to the acid bands alternating with bands of alkaline pH (Métraux and Taiz, 1979). Acid and alkaline zones are also generated along the surface of higher plant roots (Weisenseel *et al.,* 1979) in which elongation has been correlated with the acidic region (Evans and Mulkey, 1982). In *Nitella,* the wall might act as a sink for protons electrogenically pumped from the plasma membrane (Taiz *et al.,* 1981). These observations lend credence to the analogy Green (1980) has drawn between the *Nitella* cell wall and the epidermis of bipolarly elongating organs.

f) *Higher plant elongating cells*

Cell division orientation determines both the growth orientation and structural polarity of the daughter cells in obligatory fashion (Green, 1980). Control of division direction is a one-dimensional phenomenon which implies a phase of parent cell elongation followed by its separation by a cross-wall normal to the homobipolar cell axis into two cells similar in size to the original. This traditional "growth by cell division" is often considered as alternative to "growth by cell expansion". According to Green's critic "it would be better to consider cross-wall formation and cell expansion as basically separable processes and ask whether in fact an obligatory directional coupling exists".

The most reasonable immediate biophysical explanation for the elongation of growing cells lies in the properties of the side walls. Such cells which grow throughout

their length are in contrast to tip-growing cells such as fungal hyphae, pollen tubes or root hairs. Green (1980) has emphasized the stress pattern in such cylindrical cells and the role of the internal turgor pressure as driving force. He has, however, pointed out that the bipolar axis needs not to coincide with growth direction "if the tissue tensions are strong enough to pull the cell even in the direction of its cellulose alignment". From this, Green concluded that "growth direction is not always set by cell structural polarity, nor, since periclinal and T divisions exist, is division direction set by structural polarity".

The plant growth substance auxin (indole-3-acetic acid) normally accelerates elongation by increasing the plasticity of the wall (Setterfield and Bayley, 1961). A possible explanation of the formative effect in cell wall is that excessive plasticity leads to a breakdown of the transverse order in the wall. An exception to the broad correlation between transverse wall texture and elongation is, however, the outer epidermal wall of the coleoptile which has a net longitudinal microfibrillar orientation.

Auxin has been thought to stimulate cell elongation by inducing a localized H^+ secretion from cells, which causes the walls to undergo localized loosening. Auxin apparently activates (directly or indirectly) a membrane-bound proton pump (Taiz, 1984), with the result that the pH of the region of the cell wall near the plasma membrane is lowered (Rubery and Sheldrake, 1974, "chemiosmotic hypothesis", see VIII, A.2c[4]). However, the correlation wall acidification — auxin response is still uncertain (Kutschera and Briggs, 1987; A.2c[1]). Recently, it has been found that auxin also stimulated plasma membrane redox activities. This growth control by auxin is restricted to NADH oxidase linking electron flow from NADH to oxygen (Morré *et al.,* 1988). Protons and electrons which are delivered across the plasma membrane would contribute to both its polarization and the acidification of the cytoplasm and the cell wall. This has, in turn been implicated as a mechanism to stimulate the ATP-driven proton pump of the plasma membrane (Rubinstein and Stern, 1986).

The mode of action of auxin stands in contrast to the effect of a number of other plants hormones such as gibberellic acid (GA_3), kinetin, benzimidazole (BIA) and ethylene; these compounds alter the polarity of growth by changing the predominant mode of cellulose microfibril deposition with parallel changes in the distribution of microtubules. Modification of the hormone-induced effect on growth by microtubules is supported by experiments using colchicine. This confirms «the consistency with which the polarity of growth is reflected by reorganization of microtubule arrays and cellulose deposition during growth of already-formed plant organs» (Hardham, 1982).

Differential cell elongation, and consequently differential growth, is the last part of the gravitropic response (see VIII.A.2c[4]). It implicates an asymmetric release of auxin and auxin-mediated wall-loosening by enzymes such as cellulase and pectinase

on one hand, and, of course, enzymes involved in the synthesis of cellulose and other polysaccharides (Salisbury and Ross, 1985).

C. MULTIPOLAR

a) *Apiculate yeast buddings*

In addition to the usual bipolar budding, the multipolar budding that developed at different lateral sites on a single cell was observed by Goto (1982) in many apiculate yeasts of the genera *Kloeckera, Nadsonia,* etc. The rates of this multilateral budding varied with environmental conditions and yeast species, but were more frequent in acid media (pH 6.0-6.5).

As first structural basis to explain multibudding, Romano (1966) has proposed that "the spherical configuration may be due to a lesser degree of cell wall rigidity to begin with, and the appearance of sites of insufficient rigidity to withstand the internal turgor pressure of the cell may become more probable".

Genetic controlling-factors are also involved in the multibudding process. Among the many cell division cycle (CDC) mutants known in yeasts (Hartwell, 1974), temperature-sensitive strains were found to produce multibudded and multinucleate cells. The buds outgrown are grossly elongated in comparison with normal buds and this hyperpolar growth is accompanied by an apparent hyperpolarization of the cellular actin network (Pringle *et al.,* 1986).

b) *Fungal germ "multitubes"*

Multipolar outgrowth of germ tubes is a normal, genetically programmed event in the spores of many fungal species (Gottlieb, 1978). It can also be artificially induced from normally mono- (or bi-) polarized spores as those of *Botrytis cinerea* in which a microtubule inhibitor, benomyl, provokes the emergence of multiple germ tubes (Richmond and Pring, 1971). A similar effect has been observed with germinating conidia of *N. crassa* treated by antitubulin agents. However, a benomyl-resistant mutant (*bml*), mutated in its ß-tubulin gene, germinated normally in the presence of the inhibitor. These results strongly suggest that multipolar germination is due to the effect of benomyl on ß-tubulin and, by extension, that microtubule or membrane ß-tubulin is involved in the maintenance of monopolarity at conidial germination of *N. crassa* (Caesar-Ton That *et al.,* 1988).

That polarity of germ tube emergence is genetically controlled has been confirmed by the observation of multipolar germination from conidia of a respiratory deficient morphological mutant *amycelial* of *N. crassa* (Turian *et al.,* 1988). The germinating conidia of the mutant are presumptively defective in microtubule polymerization as those treated with benomyl. Significantly, both genotypic and

phenotypic conidial "multitubes" show only a faint fluorescence of rhodamine 123 revealing the low membrane potential of their dispersed mitochondria, contrarily to the bright fluorescence of the organelles frontally positioned in the single germ tube of wild type conidia (Fig. 19A; Turian and Caesar, 1987). Monopolar dominance should therefore require some type of functional complementarity between intact microtubules and respiratory competent mitochondria frontally oriented toward the thereby elected, normally single germ tube.

c) *Desmidial algae (multiradiate pattern)*

Cytomorphogenetic studies have described the extraordinary plasmatic continuity of multiradiate structure of the *Micrasterias* cell (Warris, 1950-51, uniradiate "mutants" in Kallio and Lehtonen, 1981). However, ultrastructural studies did not reveal real "plasmatic axes" (Kiermayer, 1970, in 1981) but microtubular components. Unexpectedly shaping of cells was not affected by anti-microtubule drugs (Kiermayer, 1981). Microtubules rather seem to participate in moving the nucleus as in moss caulonema (see VI.A.2d, Schnepf, 1982). Microfilaments may also be important for localizing centers of multipolar tip growth in *Micrasterias* cells (Pickett-Heaps, 1983; Waaland, 1984).

A precise regulation occurs at the sites of tip growth which is important for the expansion of the two characteristic semi-cells in these unicellular placoderm green algae. Localized ion fluxes appear to be important in the initiation of the multipolar growth centers generating the typical multi-lobed pattern. Developing semi-cells placed in an electric field showed an interesting growth elongation of the lobes toward the cathode (Brower and Giddings, 1980).

VII. POLARIZED CELL DIFFERENTIATION

Differentiation involves the structural and functional specialization of individual cells from one of a number of common basic "stem" cells which are usually competent to differentiate in several different ways. It may be regarded as essentially an *intra*cellular process involving the appearance within the cell of certain biochemically or cytologically recognizable characteristics. At its molecular basis, the process of differentiation is associated with the activation (enhancer DNA sequences) of different genes in different cells at different stages of development. Controls of gene functioning can intervene at both transcriptional and translational levels. Selective gene activation and sequential genome read out are therefore essential for differentiation.

Environments can interact with genetic mechanisms to direct the accumulation of specific cellular components to precise locations within the cell. Subsequent segregation of nuclei into these polar regions of cytoplasm results in different microenvironments for each of the genetically identical nuclei. The ensuing nucleoplasmic interactions have been shown in both plant and animal cells to be the basis for selective gene expression in different regions of cells, the developing embryo, and the mature tissues. Thus the divergence of gene expression in different cells of mature tissue is initiated by the unequal distribution of cytoplasmic components, in agreement with the famous Bünning's axiom (1952*a*) "there is no differentiation without polarity".

Unequal divisions are an early expression of polar differentiation. They define one side or corner of a cell relative to all others and are thus an expression of polarity at a cellular level (Sachs, 1974). The two unequal cells produced differ not only in size but also in the structure of their cytoplasm as seen in both light and electron microscopes (Palevitz and Hepler, 1974). Such asymmetric cell division could involve the self-assembly of the microtubules initiated by a high activator concentration directing the synthesis of the new cell wall closer to the activated site while a second center of microtubules self-assembly would be created at the opposite side of the cell (Meinhardt, 1984). In the self-regulation of unequal pattern through activator-inhibitor distribution in a linear field, the activator maximum process can regenerate: "with the removal of the activated tissue, the site of inhibitor production is removed, the remaining inhibitor decays and a new activator maximum can be triggered in the remnant tissue" (see Fig. 1.3, in Meinhardt, 1984). These divisions are found in all stages of plant development (Bloch, 1965) such as in apical cellular patterns of ferns, in the cambium and many meristemoid tissues as well as different specialized cells or idioblasts. Organisms behave according to their position and show differences between axis and direction.

According to Grant (1978), the directional organized forces in the cytoplasm produce three different types of cell polarity (Fig. 26 A):

(1) with cell organelles restricted to one region, the axis of the cell being visibly defined by that organelle accumulation; such polarity is eliminated after cutting the cell, for example that from a ciliated epithelium of respiratory tract. An hyphal apex with subtip accumulation of mitochondria would correspond to that type; (2) with oriented structures uniformly distributed throughout the cytoplasm, the cell axis being defined by their orientation; here, polarity is a property of the entire cell rather than of a region and the organelles align themselves in response to forces operating within cytoplasm; consequently, polarity is retained after cutting this cell which may originate from a muscle in which longitudinal myofibrils have the same polarity as that of the surrounding cytoplasm; (3) based on intracellular gradient either of organelles or of metabolic systems with peaks of activity at one end diminishing gradually toward the other. When a high point metabolism appears at one pole, with a decrease toward the opposite pole, inversely "double gradients" may appear as the yolk-pigment, reverse distribution of the frog egg where yolk is concentrated at the vegetal pole while pigment is highest at the opposite animal pole.

A. INTERCALARY DIFFERENTIATIONS

These are primitive types of differentiation which are controlled by three different kinds of mechanisms (Bonner, 1974): (1) production of substances, (2) time of appearance of certain substances, and (3) spacing of the substances that is, their localization. Bacterial spore division examplifies the *temporal* kind in which there is a change in time only, while the *spatial* one intervenes at heterocyst formation in blue green "algae" or Cyanobacteria.

1. *Bacterial endospores*

Endospores, formed inside a bacterial cell, are the resistant forms of many bacteria; they are non-dividing, dormant cells, more resistant to desiccation, heat and other adverse conditions than the vegetative organisms. While bacterial vegetative cell division is characterized by the symmetric septation, spore formation implicates an asymmetric septation. This asymmetric septation during cell division has been regarded as an ideal model system for studying the mechanism of cell differentiation (Halvorson, 1965; Aronson and Fitz-James, 1976).

Induction of endosporulation and hence the sporulation "division" is apparently coupled to the cell division cycle at an early point in DNA replication (Hitchins, 1978). When the early stages of this complex phenomenon are considered

as equivalent to a modified cell division (Hitchins and Slepecky, 1969) directs it at the problem of the control of the topological relationship between the septum and the genome in cell division. Therefore, bacterial sporulation can be viewed as a general problem of the topology and polarity of DNA segregation (Hitchins, 1978) and as such has been considered as a model system for studying cell polarity and patterning.

Every sporogenic cell is polar (Fig. 25 A) so that asymmetric septation creates cells with different composition. The stage of partial engulfment of the pre-spore cell precedes that of the completely engulfed cell compartment or forespore surrounded by mother cell cytoplasm (Freese and Heinze, 1984). The cell is irreversibly committed to continue its differentiation when forespore engulfment is completed by a double membrane. The inner membrane has the normal orientation of a membrane surrounding a cell's cytoplasm. In contrast, the outer forespore membrane resulting from the engulfment process should have opposite polarity (Freese, 1981). Consequently, compounds whose active transport depends on the membrane polarity should not be actively transported into the forespore; facilitated transport is possible only when concentration of ions is different in the two cell compartments (Freese and Heinze, 1984).

Factors such as the ionic composition of the growth medium can influence cell polarity in *Bacillus subtilis*. They have been statistically analyzed by Dunn (1980) who has shown that, within chains of four sporangia or quads, "the probability that a spore is formed distally in relation to the newest division septum is used as a measure of cell polarity; then a cell's polarity is influenced by the behaviour of its sister sporangium (i.e. sister cells do not position their spores independently), but not by its position within a quad". After having recorded the positions of spores within the sporangia of a quad, Dunn could ask the following questions: (1) Is the polarity of a cell that gives rise to a type α-sporangium different from that giving rise to one of type β? That is, is there any effect of sporangial position within a quad on polarity? (2) Are sister sporangia interdependent or do they position their spores independently? For example, is there any influence of a type α-sporangium on its sister spores type β-sporangium, or vice versa? According to Dunn (1980) there is no difference in the polarity of the sister cells that give rise to type α or type β sporangia, even though the sister cells were found to some extent to be interdependent.

2. *Cyanobacterial heterocysts*

The differentiation of a cyanobacterial vegetative cell into a specialized, nitrogen-fixing cell, called heterocyst, generally occurs by intercalary, asymmetrical division of one of a few swollen precursor cells or proheterocysts. This developmental process is both temporally and spatially controlled (Carr and Whitton, 1982). It

resembles sporulation in its requirement for the orderly expression of sets of genes; moreover, two different rearrangements occur near the nitrogen fixation (*nif*) genes (Golden and Wiest, 1988). However, the differentiation of heterocysts differs from simple sporulation by its irreversibility even upon addition of an organic nitrogen source.

Anabaena, one of a number of species of filamentous cyanobacteria, differentiates its heterocysts at regular intervals along each filament in response to deprivation of a combined nitrogen source under aerobic conditions. The vegetative-cell functions of photosynthetic oxygen evolution and CO_2 fixation are shut down; in heterocysts, they are replaced by cyclic photophosphorylation and oxidative carbon metabolism yielding ATP and reductant for the anaerobic process of nitrogen fixation, its ultimate product.

The big questions about heterocyst differentiation have been posed at the cellular and molecular levels. Concerning the former, little is known of the rules governing the selection of vegetative cells for differentiation. In an already-differentiated filament growing on N_2, the vegetative cell placed midway between two existing heterocysts is the most likely to differentiate next. That is thought to be at the lowest point in a gradient of inhibitor flowing from the heterocyst (Wolk, 1967). Glutamine is a good candidate for the inhibitor. In the case of the transition from growth on combined nitrogen to growth on N_2, much less is known about the selection of cells for differentiation. In some species the slightly smaller daughter cell produced by asymmetric division may be selected (Wilcox *et al.,* 1975). It is possible that the candidate cells have just completed chromosome replication. The factors that govern the selection of vegetative cells for differentiation, and which determine the pattern of heterocyst spacing, thus remain mostly unknown (Haselkorn *et al.,* 1978).

B. APICAL DIFFERENTIATIONS

1. *Monopolar patterns*

a) *Fungal exosporulation*

Axial polarity implying both a clear axis and a clear direction (Bloch, 1965) precedes the asymmetrical division intervening on the apices of elongated structures. When differentiation concerns uniformly only one end of the axis can we speak of apical *mono*polar differentiation. It is examplified by the differentiation of conidia or of zoosporangia on the apices of specialized hyphae, the conidiophores or the sporangiophores respectively. By contrast, when this same end is differentiated into two different structures such as male and female gametangia in the *Allomyces,* can we speak of apical *bi*polar differentiation (*bi*polar axiation).

a[1] *Conidia*

Just as the key to hyphal growth lies at the tip (VI.A.2b) comprehension of blastic and thallic conidium initiation is suggested to be linked to pivotal events which occur at the hyphal tip and lead to the formation of one or more swollen propagules. At the vegetative-differentiation transition, when hyphal tip growth is arrested, the polar distribution of organelles, with its tip zone of mitochondrial exclusion, is dissipated and the Spitzenkörper disaggregates (Najim and Turian, 1979; Cole, 1986).

The two principal types of conidium ontogeny are referred to as "blastic" and "thallic" developments. Blastic conidia differentiate apically or laterally from a fertile hypha by the blowing out and de novo growth of part of the hyphal element and are delimited from the parent hypha by basal septum. Blastic conidia commonly secede from their parent hypha by the centripetal splitting of this same basal septum, a process referred to as schizolysis. Turian (1976*b*) has demonstrated that macroconidiation in the *Monilia* state of *Neurospora crassa* can be manipulated under experimental growth conditions so that both blastic and thallic modes of conidium ontogeny occur along aerial chains arising from the same mycelium. This process corresponds to a blastoarthric type of development (Turian and Bianchi, 1972).

By contrast, in the phialidic type of development, conidia are basipetally budded from a mother cell, the phialide and, therefore, the youngest conidium is closest to the fertile apex of the phialide. Deviations in the normal, phialoconidial ontogeny of *Penicillia* and *Aspergilli* have been observed such as the mutational change from basipetal (phialidic-type) to acropetal (blastic-type) cell proliferation. The conidial chain elongation becomes acropetal, blastosporogenic in an "abacus" mutant of *Aspergillus nidulans* (Clutterbuck, 1969), and the normally basipetal differentiation of phialoconidia is blocked at nonpermissive conditions in a temperature-sensitive and osmotic-remedial mutant of *A. aureolatus* (Vujicic and Muntanjola-Cvetkovic, 1973). To account for the observed reversion of the young phialide-like element at permissive temperatures, the defect could be sought in the wall of the mutant (Vujicic and Muntanjola-Cvetkovic, 1973) or in the nuclear differentiation in the phialide of *A. niger* (Raper and Fennell, 1965). In *Penicillium claviforme,* the change from basipetal to acropetal cell proliferation was more clearly ascribed to the reversal of a cytoplasmic basophilic (RNA) gradient leading to an altered behaviour of daughter nuclei (Zachariah and Metitiri, 1971).

That the strength of polar differentiation of conidia is genetically-controlled is examplifed by conidia budding from the tips of long, aerial hyphae in wild type of *Neurospora crassa,* contrasting with the premature conidiation of short conidiophores in "crisp" mutants (Turian and Bianchi, 1972).

Microtubules are some way involved in conidiation of *Aspergillus nidulans* as shown by the morphogenetic consequences of selective tubulin mutations; conidiation-resistant mutants were able to conidiate in the presence of benomyl,

strongly suggesting that β_3-tubulin functions during spore formation (Morris *et al.*, 1984).

a^2 *Sporangia*

Structural changes accompany sporangial development in the "water moulds". During spore formation, the sporangium enters a brief phase when the cleavage furrows between spore initials become obscured, and under the light microscope the whole protoplasm takes on a uniform slightly granular appearance. This phase is termed the homogeneous phase (H-phase, Busgen, 1882, see Money and Brownlee, 1987). At the H-phase, spore delimitation is completed as the network of cleavage furrows in the sporangial cytoplasm fuses with the plasmalemma to produce continuous membrane-bound channels between the central vacuole and periplasmic space (Gay and Greenwood, 1966).

Coincident with the H-phase, in species of *Achlya* and *Saprolegnia,* the sporangial volume decreases by up to 13% and the previously concave sporangium-delimiting septum flexes into sporangium (Gay and Greenwood, 1966). These phenomena suggest that the sporangial pressure potential decreases during the H-phase as the tonoplast and plasmalemma are broken. Using an extracellular vibrating probe, Armbruster and Weisenseel (1983) measured an inward positive current at the tip of pre-H-phase sporangia of *Achlya debaryana,* which was replaced by a biphasic outward current of 2.5 $\mu A\ cm^{-2}$ during the H-phase. The inward current was subsequently restored. A similar collection of physiological changes accompanies the completion of cleavage in other aquatic fungi. Current patterns similar to those measured by Armbruster and Weisenseel (1983), including current efflux during cleavage, have been measured around thalli of *Blastocladiella emersonii* (Stump *et al.,* 1980). New results obtained with *A. debaryana* (Money and Brownlee, 1987) suggest that at least part of this current is carried by K^+ efflux. The significance of this inversed current patterns for differentiation from hyphae is still controversial (Harold *et al.,* 1987). A developmentally regulated enzyme, neutral protease, loses its affinity for Ca^{2+} ions at the transition vegetative — zoosporogenic hyphae in *Allomyces arbuscula* (Ojha and Turian, 1985).

a^3 *Basidiospores*

Basidiospores formation in higher Fungi involves cytoplasmic and wall layer synthesis and their interaction. Sterigmata appear to result from renewed tip growth at restricted loci in the basidial apex (McLaughlin, 1973). Basidiospore initiation is signalled by asymmetric sterigma tip expansion and it elongates by a mechanism homologous with hyphal tip polar growth (McLaughlin, 1977; Oláh and Reisinger, 1981). Sterigma outgrown from the apical dome of the basidium contain a dense cytoplasm with a cluster of vesicles near their tip. These must contribute to the polar

elongation of the usually four sterigma which reach their fullest length before spore initiation. Their wall is continuous with that of the basidium, but is thinner (bi- instead of four-layered).

b) *Algal exosporulation*

The polarized organization of vegetative filaments of the yellow green fresh water alga *Vaucheria* is lost at spore induction. The first indication that a vegetative filament is about to differentiate into a zoosporangium is a gradual darkening of its tip by migrating cytoplasmic organelles as they displace the large central vacuole. Many nuclei are involved in that polarized process; they accumulate through mass movement into the developing sporangia after release from the active microtubular band of the zone of cyclosis. Chloroplasts also flow into this region when the tip of the filament begins to enlarge. As in Fungi (a), there parallely occurs a dissipation of the gradient of vesicles in the enlarging tips of vegetative filaments arrested in their elongation. Soon after migration and accumulation of nuclei and chloroplasts into the filament tip, the pairs of centrioles associated with each nucleus begin to form flagella within internal flagellar vesicles. The flagella and their nuclei converge into many internal flagellar pools which then polarly migrate to the surface of the zoospore and become part of the plasma membrane. These events in the filament apex take place during the septation of the vegetative filament. At the time of zoospore release, all nuclei are positioned just beneath the zoospore surface and are intimately associated with pairs of external flagella (Ott and Brown, 1974b).

Positional control of algal differentiation has been reviewed in 1984 by Waaland who referred to Kataoka's work (1975) for tip organization in *Vaucheria* in which branching was induced by unilateral illumination.

2. *Bipolar patterns*

a) *Mating types*

The bipolar sexualization in the *Allomyces* generative of sexually compatible gametes corresponds to a homothallic condition. By contrast, in dimictic or heterothallic forms of Fungi, a haploid individual can never breed with more than 50% of the whole population but can always mate with half the progeny of the same zygote (Burnett, 1975). Such mating systems thus favour the opportunity for outcrossing in preventing the selfing of a single haploid. They are functioning through a unifactorial, bipolar expression of one of the two alleles A/a at the same locus as best illustrated by the classical, heterothallic *Neurospora crassa* and *N. sitophila* (Whitehouse, 1949).

Those phenomena described as bipolar mating systems correspond to homogenic incompatibility defined by Esser and Blaich (1973) as the inhibition of zygote formation between partners of the same species due to the heterogeneity (pluripolarity)

of their incompatibility loci. Such a situation has been analyzed in different strains of *Podospora anserina* and appears to extend to other fungal species.

Relatively little is still known about the chemical basis of the bipolar compatibility versus incompatibility of heterothallic Ascomycetes. A complementary mechanism involving A and a mating gene products acting as the "key-lock system" has been envisaged for zygote formation (Esser and Kuenen, 1967); this assumption implies that in the incompatibility combination, both partners form gene-like products unable to complement.

Bipolar compatibilities or incompatibilities are independently controlled of the sexual phenotypic traits, as examplified by the heterothallic *Neurospora* spp. where a female coiled structure, the ascogonium, can statistically be either of the (+) or (−) mating type and thus conjugates with (−) or (+) conidia respectively (Turian, 1978*b*). This bipolar compatibility or mating system is also effective in Zygomycetes (Mucorales +/−), the yeasts (α/a) and many higher Fungi (A/a). However, an exceptional situation, the *tetra*polarity, can be preeminent in certain species of these higher Fungi, as examplified by the genetically well-known "split gills" *Schizophyllum commune.* Such tetrapolarity is controlled by two allelic pairs designated as Aa and Bb. Full compatibility and fertility (fruiting bodies) is only assumed by the fully complementary formula AaBb (Raper, 1966).

The simpler bipolar system of yeasts controlling transcription-translation of mating type substances is not fully fixed in homothallic strains. It is controlled by the changing expression of mating type substances known as polypeptides (Herskowitz, 1987). In their vegetative phase, yeasts are predominantly diploid cells heterozygous for the mating-type locus MAT. In heterothallic strains, germination and cell division result in clones of stable haploids of either *a* or α mating type. However, in homothallic strains a single a or α spore will rapidly give rise to *a*/α diploid without having to encounter cells of different clonal origin and mating type. When germinated in isolation from other cells, homothallic spores with α mating type, for example, divide by budding to produce a mother cell and a daughter cell, both of which retain the original α mating type. During their subsequent cell divisions, the mother cell switches mating type and produces a pair of a cells, whereas the daughter retains its mating type and produces a pair of α cells. Cells of opposite mating type now arrest each other in G_1 by secreting one pheromone (*a* or α) and by responding to the other (see Saier, and Jacobson, 1984). During the pheromone-induced G_1 arrest, the cells elongate, becoming pear-shaped ("schmooing", Fig. 18 B) and eventually fuse to produce a pair of diploids now heterozygous while recovering an ovoid cellular shape to further divide by budding during the diplophase (Cross *et al.,* 1988).

It remains the question of how does a cell divide to produce daughter cells which have different developmental fates? According to Nasmyth and Shore (1987), the sequence of developmental events are regulated at the level of transcription factors

encoded by *Switching* (*SWI*) genes which activate an endonuclease gene (*HO*) at a precise stage in the cell cycle of mother cells. Since *HO* is only activated in one of the sister cells after division (the mother), adjacent cells of opposite mating type are generated which respond to each others' secreted pheromones by inducing genes involved in conjugation and this leads to the formation of a diploid.

b) *Sexual disjunction*

Rigid rules govern the placement of reproductive organs in lower plants. In mosses, with the exception of *Sphagnum,* the antheridia and archegonia are formed at the tip of the stem but not by the apical cell itself. Interestingly, in conifers the position of male and female cones is also subject to relatively strict rules: at the onset of its sexual maturity, *Pinus sylvestris* initially produces some female cones on leading shoots; male cones are then produced basitonically on lower branches, female cones being produced acrotonically (Carr, 1984). These positional rules in conifers are maintained even when cones are induced experimentally by treatment by gibberellins (Pharis, 1978).

In higher plants, phyllotaxis increases in complexity with the onset of flowering (Carr, 1984). The same apex can produce first a suite of leaves, then a closely-set suite of flowers and subsequently reverse to leave production. Informational controls regulate this reciprocal positioning of floral parts and leaf primordia. There are also examples of anisophylly and anisoclady (see in Carr, 1984). Positioning of stamens (androceum) relative to that of the pistil (gynaeceum) is described in classical botany as either epigynous or hypogynous.

A similar positional pattern is shown by the gametangia of the aquatic molds of the genus *Allomyces.* In that most experimentally studied system, the bipolar phenotypic disjunction of two sexually distinct multinucleate cytoplasmic "territories" (coenocytes) in the apices of the gametophytic haploid hyphae is reminiscent of the unequal division described by Bünning (1957) as a basic mechanism of cell differentiation. The fact that the bipolar axis is specifically (genetically) oriented, as shown by the epigynous *versus* hypogynous arrangement of the gametangial couples in *A. macrogynus* versus *A. arbuscula* (Emerson, 1955) adds to the interest not to speak of the complexity of the case (see Plate, p. 3).

As first step in this double differentiation, dissipation of the monopolar organization of the vegetative hyphal apices precedes the specifically oriented, gradiential reorganization in the enlarging, now club-like hyphal apices (Turian, 1969). The direction of such a chemo-structural gradient should therefore be under genetical control, namely that of some bit of DNA considered as the positional information. The transcription of this information is initially triggered — and the positioning gene(s) switched-on — by environmental stimuli such as starvation in aerated liquid media.

Out of its apparent cytoplasmic uniformity at inception, double differentiation proceeds by the inversely oriented sorting out of the characteristic, functional structures of male and female cells. This DNA-controlled positioning process (Fig. 24 C; Ojha and Turian, 1971) can tentatively be grasped from both extremities of its span: (1) at the start, with the DNA and its positioning effect on cytoplasmic effectors, leading to a polarized expression of the sexual information; (2) at termination, with the characteristic differential features of the genetic expression, the four most prominent features known which contribute positively or negatively to the sorting out of male from female gametangia, being (Turian, 1975): (1) the nuclei (also nucleoli), smaller and more numerous in the male gametangia; (2) the ribosomal nuclear caps, more massive and with more RNA in the female gametangia; (3) the mitochondria, apparently less numerous and often poorly cristated in the male gametangia; and (4) the orange pigment (γ-carotene) only in the lipid granules of the male gametangia. The larger size of the female gametangia reflects their more efficient synthetic power.

It has been suggested that it is an AT-rich cytoplasmic DNA which could function as positioning DNA by orienting a basophilic, RNA-proteinic gradient cytochemically detected along the axis of the differentiating gametangia (gradient head in the female "territory") as most visibly expressed at their nuclear cap forming stage (Fig. 24 A; Turian, 1969). Such massive concentration of ribosomal RNA in the female gametangia and the free gametes was confirmed by their twice higher RNA/DNA ratios compared to male cells. To meet the need for more energy to produce a RNA and protein richer female cell should request efficient mitochondria. Those were shown to be more internally developed (cristae) in female cells (Turian, 1969). Additional evidence for the oxidative deficiency of "male mitochondria" came from cytochrome oxidase measurements and the masculinizing effects of acridine compounds (antimitochondrial agents) by suppression of the female gametangial differentiation (Turian, 1975; Olson, 1984).

A dynamic scheme involving gene-controlled polar liberation in the hyphal cytoplasm of episome-like factors activating mitochondrial competence in presumptive female zone has been proposed to integrate all available genotypic and phenoptypic data concerning this polarized sexual disjunction (Fig. 24 B; Turian, 1969, 1975). Superposed on the increased respiratory competence developed by the "feminizing mitochondria" in response to oxygen tension, there occurs a gene-induced oxidative deficiency of the mitochondria present in the presumptive male "territory", through the acridine-like action of a polarly distributed suppressive factor. Triggered activation of the positional DNA would be necessary to insure such polarized spreading of the suppressive masculinizing factor. The "mas" gene of a male mutant of *A. arbuscula* could well be the gene producing the suppressive factor and the «pol» gene the true positioning DNA to achieve epi-*versus* hypogyny. The control of a polar

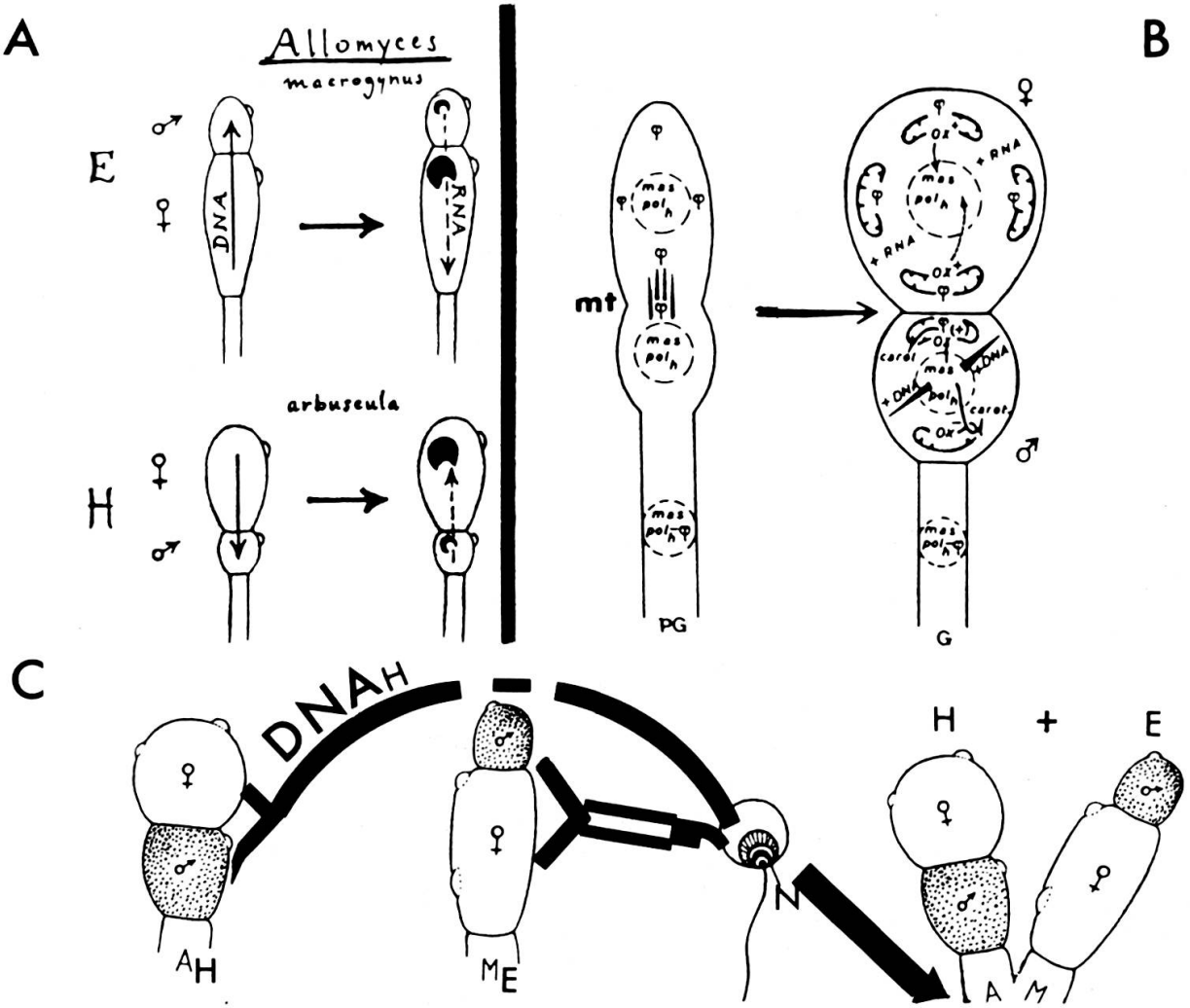


FIG. 24.

Polar positioning of *Allomyces* gametangia.

(A) Specifically inverse bipolar gradiential segregation of DNA and RNA at gametangial differentiation of the epi (E) *versus* hypogynous (H) species of *Allomyces*. "Male-head" gradients of DNA content (Feulgen-Giemsa stainings) — mitogenic capability are first produced (left), followed by the inverse "female-head" gradients of RNA content (toluidine blue staining) expressed in their larger nuclear caps ("ribosomal-crescent bags") at the pregametic stages. From Turian, The Nucleus, 1961.

(B) Model of the biochemical events occurring at the bipolar segregation of male and female gametangial cytoplasms founded on (1) the masculinizing effect of acridines or of presumed similarly natural compound(s) acting as suppressors of feminizing (φ-) prooxidative factors favoring an increase of the RNA/DNA ratios in the female gametangia (see A); (2) the polar positioning of the suppressive factor(s) along tracks of microtubules (mt)-microfilaments between the progametangia (PG); the antitubular - antifilament compounds dissipate such polar segregation, and, spreading the female suppressor(s), masculinize the gametangial (G) zone. From Turian, 1969 and Turian and Ojha, 1987.

(C) Transfer of positional DNA from the normally 100% hypogynous (H) species *A. arbuscula* to the normally 100% epigynous (E) *A. macrogynus* grown and sexually differentiated from the wall-less meiospores (N = nucleus) germinated in the presence of the heterologous DNA_H. The ratios of hypogenous *versus* epigynous gametangial arrangements differentiated from the same main hypha (see right of the Fig. C) was the highest in the DNA transfer direction from $_H$ to $_E$. Reciprocal DNA-positioning effects — from DNA_E — were less efficient. The highest ratios obtained corresponded to those shown by both the natural hybrid *A. javanicus* ($_H \times _E$) and certain strains issued from *in vitro* hybridizations (Emerson, 1955) spanning from nearly fully masculinized to nearly fully feminized strains through bisexual hybrids bearing inversely positioned gametangial couples. Dotted gametangia are γ-carotene - pigmented males (*carot.* in B). Adapted from Ojha and Turian, 1971.

disjunction of gametangial territories could thus be effected through the gradiential segregation of a respiration inhibitory factor.

The chemo-mechanical mediators of genetical polarity in the *Allomyces* being still unkown and, in view of the recent biochemical trend to attribute such a role to the cytoskeleton (Quatrano, 1978; Fulton, 1984), it became tempting to ascribe the vectorial cytoplasmic movement to microfibrillar-microtubular elements of the cytoskeleton. Recently, it has been found that the genetically-controlled inverse bipolar sexualizing gradient of the *Allomyces* can be dissipated by adequate concentrations of the antiactin cytochalasin E or the antitubulins DMSO and benlate (Turian and Ojha, 1987). These effects suggest that the presumed disruption of bipolarizing tracks allows the dominant male tendency (suppressive effect) to overflow the presumptive female "territories" in the sexually differentiating hyphal apices of *both* epi- and hypogynous species. A first conclusion was that "DNA-controlled inverse bipolarity is mediated by the inversely-directed function of filamentous actin and/or tubulin microstructures vectorially conveying the sex-determining elicitors".

C. APICO-BASAL DIFFERENTIATIONS — HETEROBIPOLAR AXIATION

There are many instances in plants (see Miller and Bassel, 1980) in which cell differentiation is initiated by the establishment of a polar axis preceding the unequal cell division occurring transverse to that axis (Fig. 26).

Cell differences arise as result of influences, possibly electric potential gradients which bring down unequal distribution of cytoplasmic factors in the mother cell before that division occurs. Electrical potential differences, resulting in the electrophoresis of information molecules, are thus important in bipolarity and unequal division in eggs and others cells (Smith and Grierson, 1982).

Issued from a geometrically asymmetric cell division, the daughter cells are of different sizes and develop directly into different cell types. Bünning (1957) described several examples of this phenomenon and advanced what is probably the most widely-held view of the morphogenetic function of asymmetric cell division. This hypothesis states that there is polar cytoplasmic differentiation within the mother cell. When the nucleus migrates to one end of the cell, and an asymmetric cell division occurs, the two resulting nuclei reside in different cytoplasms. Since the cytoplasmic environment regulates which genes are expressed and which are repressed at a given time, the different cytoplasmic environments of the two nuclei call forth the expression of different genetic informations from each and result in cellular differentiation.

Some important questions exist concerning the detailed operation of this hypothetical scheme for the induction of cellular differentiation. Two major points are: what is the origin of polarity in the mother cell; and why is geometrical asymmetry of cytokinesis an obligate step in so many instances? Polarity may exist in the mother

cell as a preexisting, persistant state, or it may arise epigenetically in a mother cell which initially is non-polar. An epigenetic origin of polarity appears to be the situation, for example, in zygotes of the Fucales. The egg is apolar, whereas a polar axis may be established in the zygote after fertilization by the imposition of any of several external gradients — for example, in pH, ionic concentration, electrical field or light intensity (Jaffe 1968, 1970; Quatrano, 1978). This axis determines a polarized redistribution of cellular organelles, and the mitotic spindle is oriented so that cell division occurs transverse to the polar axis (Fig. 20 A). The cell at one pole of the axis develops into a multicellular rhizoid, whereas the other cell and its derivatives become the thallus. In contrast to this situation, one may conceive of a cell which has a pre-existing polar axis — perhaps having a structural basis — which is persistant and relatively unaffected by external conditions.

The molecules or particles accumulated in one cell could be of two types: (a) cell-specific components which immediately distinguish the new cell from its parents, or (b) components of a more "determinative" nature which would commit the new cell and possibly its progeny to a particular developmental pathway, ultimately leading to the expression of a specific cell or tissue type.

1. *Caulobacterial cells (flagellate-stalk poles)*

A unique feature of these so-called prosthecate bacteria is that they carry out obligatory well-defined differentiation changes during their dimorphic cell cycle. A new cycle is initiated with a new round of chromosome duplication by the first type of so-called stalked cell; their stalk or prostheca is a polar extension of the lipopolysaccharide and mucopeptide layer of the cell wall which provides attachment of the cell for some suitable substrate. After the main body of the cell has enlarged to form the predivisional cell an asymmetric division follows to give one daughter cell that retains the stalk and another which develops a flagellum and fimbriae becoming a swarmer cell.

Under normal growth conditions, a new stalked cell synthesizes an intracytoplasmic structure at the pole opposite the stalk, and this process represents the first step in a cycle of polar differentiation. It has been suggested that this structure is a membranous organelle that can be distinguished from cell mesosomes by the lack of continuity with the cytoplasmic membrane. The same type of membranous organelle is found at the stalked pole of the mature cell. We are still unsure of the nature of this organelle, because electron microscopic analysis does not consistently reveal a discrete, membranous structure in the region of the polar organelle.

The second occurrence in the differentiation process is the formation of a single flagellum and several pili also at the pole opposite the existing stalk, thus generating a typically bipolar pattern. Binary fission yields a sessile stalked cell and a motile swarmer cell (Fig. 25 B). The daughter swarmer cell must differentiate into a stalked

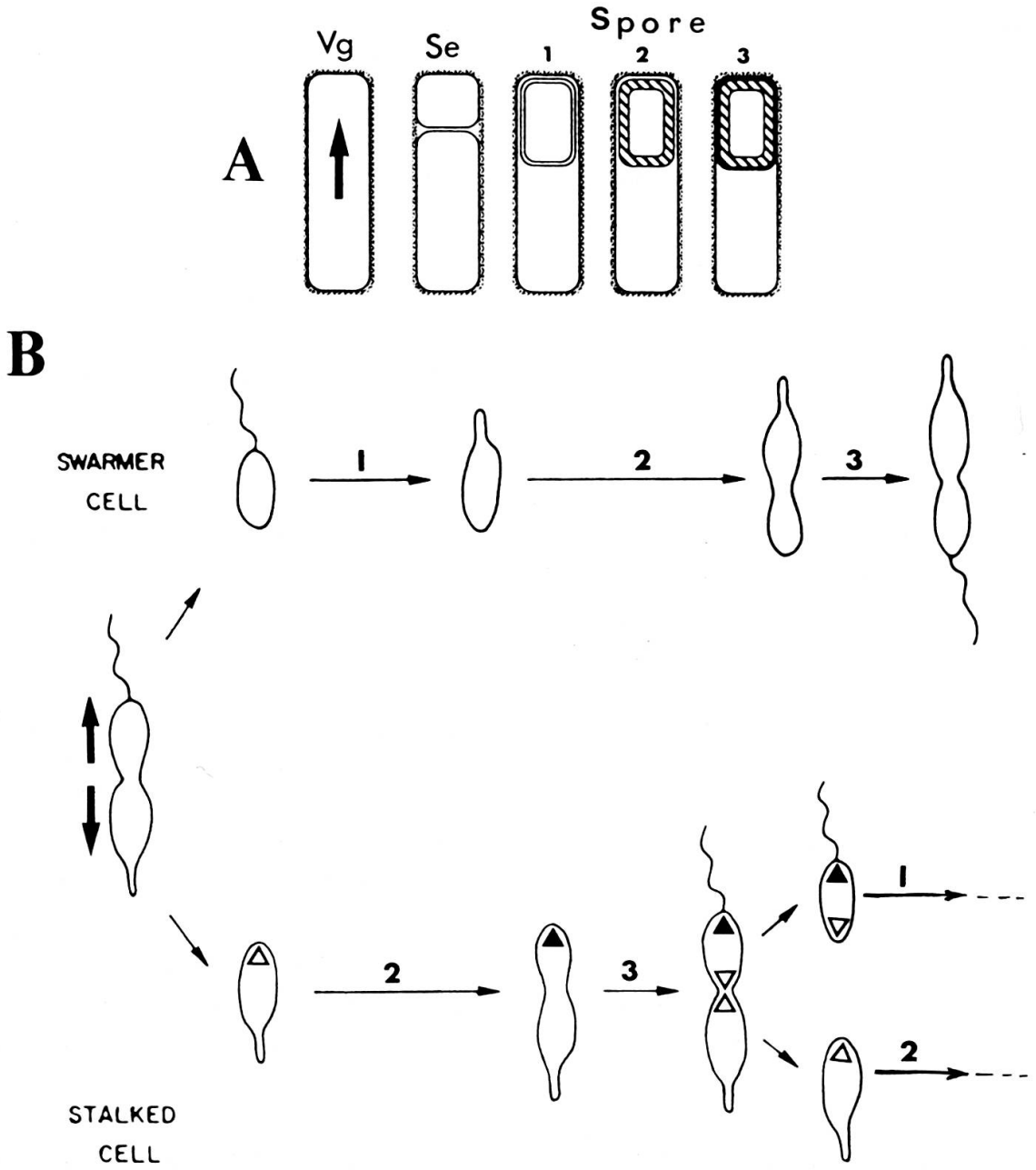


FIG. 25.

Polar differentiations in Bacteria.

(A) Monopolar differentiation at *Bacillus* sporulation: asymmetrical division (arrow) of the undifferentiated vegetative cell (Vg); septation (Se) followed by spore differentiation from the protoplast stage (1) through cortex (2) to coat formation and maturation (3). Adapted from J. Errington, Nature 333: 399 (1988).

(B) Bipolar differentiation in *Caulobacter crescentus:* asymmetric cell division (opposite arrows) producing the swarmer cell and the stalked cell. Repeated divisions of the stalked cell produce flagellated swarmer cells (2, 3); the former cells are then converted (1) to stalked cells. Hypothetical organizational centres (open triangles), laid down in division, become sites for polar assembly (closed triangles) in the next cell cycle. Adapted from Newton *et al.,* 1985.

cell before it is capable of cell division. This transition, the third step in the differentiation process, involves the deposition of new cell wall material at the point of attachment of the flagellum to the cell. The preexisting polar membrane structure now constitutes the interior of the newly forming stalk, where the flagellum can be found as an appendage at the tip of the stalk (Shapiro *et al.*, 1971).

The two cell types of the dimorphic cell cycle are out of phase in their cell cycles: the stalked cells commence a new cycle immediately with the initiation of a new round of chromosome replication; the swarmer cells, however, are immature and of necessity are required to undergo some as yet unknown differentiating steps before becoming stalked mother cells and embarking on division. Most obvious is the repression of DNA replication in these cells, with the event proceeding only when stalk development has taken place some 30 minutes after cell division.

Several features of developmental regulation in *C. crescentus* distinguish it from other systems. First, a "terminally" differentiated cell type is not formed; the swarmer cell is not some kind of a motile spore, since it is metabolically active and grows in preparation for the initiation of DNA synthesis. Second, culture conditions, such as nutrient deprivation, are not required to trigger any of the developmental stages; and third, cell-cell interactions have not been observed to play a role in development. The dimorphic cell cycle in *C. crescentus* cell represents a "stripped down" version of cell differentiation in which the temporal and spatial events are cell autonomous and occur repeatedly as part of a vegetative cell cycle (Newton *et al.*, 1985).

The two progeny cells are differentiated with respect to cell structure, but also they are programmed to follow different cell cycles; the stalked cell initiates DNA synthesis immediately after division, whereas the swarmer cell enters a presynthetic gap (G1) required for differentiation into a stalked cell. The pattern of protein synthesis in the two cells is also different and organization of the predivisional cell involves signals for positioning of proteins to one portion of the cell opposed to another (Shapiro, 1985).

A functional differentiation of polar membrane domains has been described by Newton *et al.* (1985): the assembly of the surface structures on the incipient swarmer cell always occurs at the new cell pole, and the mutational analysis outlined above showed that formation of the assembly sites requires completion of a late step(s) in cell division; the position of these sites is specified by "organizational centers" that are laid down as part of the division site in the cell cycle before assembly is initiated. As a consequence of these events, the new cell pole should be stably differentiated from the remainder of the cell envelope.

An important problem in localized assembly is to identify the stage of translocation at which the subunits of the surface structures are targeted to the polar membrane domain. Pulse-chase experiments have shown that newly synthesized flagellin in *C. crescentus* cells is translocated for assembly in the sequence soluble pool — mem-

brane pool — assembled filament. In very short pulse-labelling experiments, followed by radioimmunoassay for labelled flagellin in polar and non polar vesicles, almost the entire membrane pool flagellin was found in the polar vesicles. Thus, flagellin is apparently targeted to the cell pole as a relatively early stage of translocation to the membrane (Newton *et al.*, 1985).

Recently, monoclonal antibodies have been isolated (Sommer *et al.*, 1986) that bind either uniformly to the cell surface or to one of the cell poles. The antibodies specific for proteins at the flagellated cell pole and at the stalked cell pole verify the presence of stable membrane domains in the intact cell. These antibodies are being used as probe to study the mechanism of polar membrane protein translocation.

Mutants that are blocked at various stages of morphogenesis offer a promising approach toward understanding the relation between gene function and the concerted series of events that eventually results in bipolar differentiation.

2. *Fungal cells (rhizoid-hyphal poles)*

A flagellated spore of an aquatic Phycomycete such as *Allomyces* spp., whether a zoospore, meiospore or zygote, withdraws its flagellum(a), rounds up, and encysts (Olson, 1984). At cyst germination, a germ tube grows out from one pole of the cyst and rapidly branches to establish the rhizoidal system. Following the establishment of that rhizoidal system, a second much broader germ tube — in which flows an inward positive electric current (Youatt *et al.*, 1988) — grows out of the cyst at the pole opposite (180°) the point of origin of the rhizoidal system (Fig. 26 B). This second germ tube forms the basal cell of the thallus which then branches distally into dichotomous hyphae (Turian, 1985*b*). In *Allomyces* germlings, the hyphal pole can be deviated from its opposite positioning (normal bipolarity) by mechanical (centrifugation) or chemical (cycloheximide) treatments (Turian, 1958, 1962). This could result from a disruption of the cytoskeleton (protein?)-controlled bipolar axiation.

3. *Algal cells*

a) *Eggs (rhizoid-thallic poles)*

A basic part of algal pattern formation is the development of polarity which is itself closely connected to the formation of electrical and chemical gradients. The Fucales have provided classical materials for studies of the embryogeny of seaweeds and more is known about the ova eggs and young embryos of these plants than of any other group. They have afforded botanists materials which are comparable in many respects with the eggs of various amphibians (VII.C.6a[5]).

The fertilized egg of *Fucus* is a spherical and relatively undifferentiated body. It contains homogeneously distributed brownish green photosynthetic plastids and is naked before fertilization; but immediately after there is a secretion of mucilage

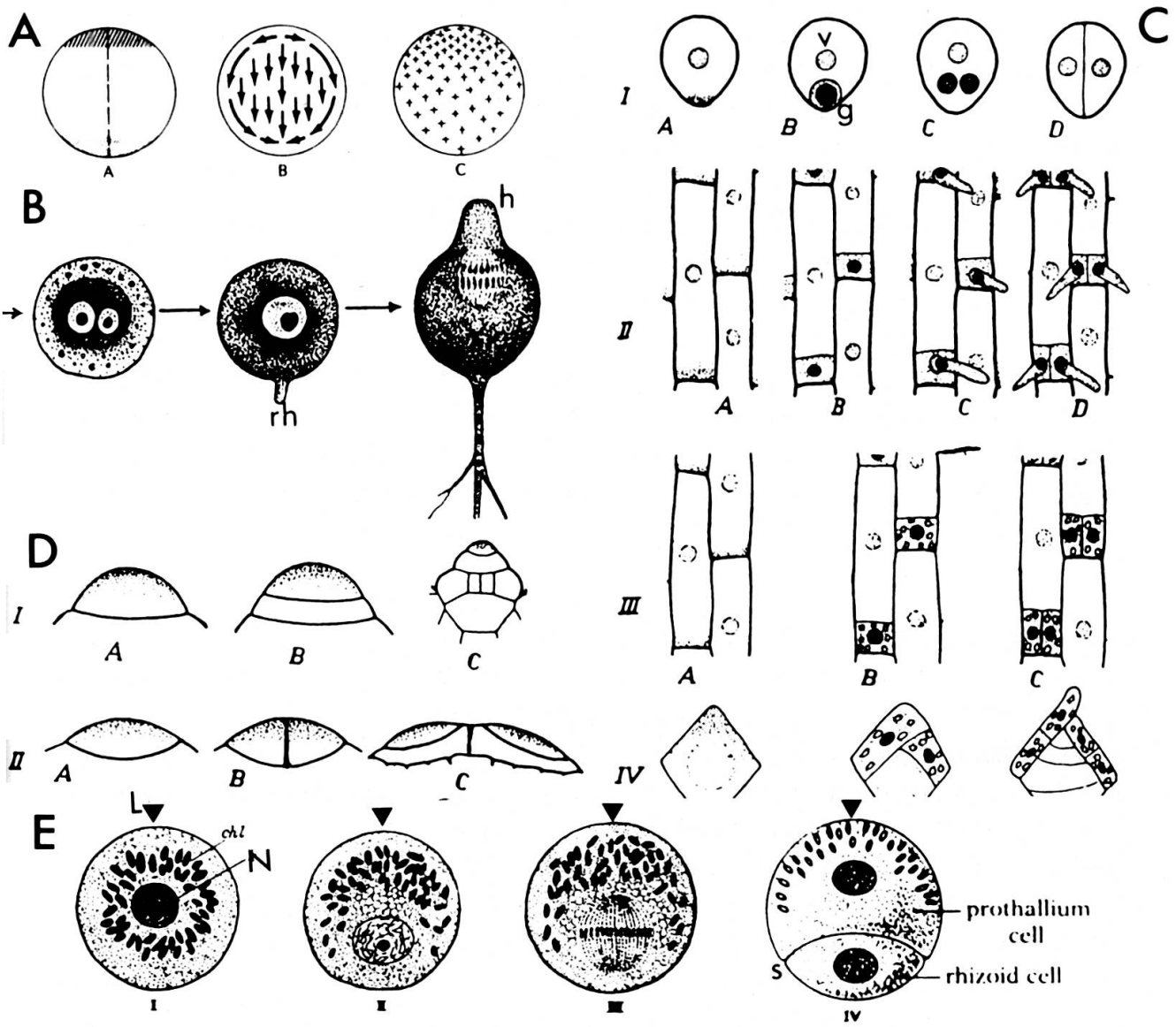


FIG. 26.

Cell asymmetry generative of bipolar differentiations.

(A) Types of cell polarization: (A) polar field polarity; (B) structural orientation polarity; (C) gradient polarity. From Grant, 1978.

(B) Gradient polarity with internal reorientation of organelles (mitochondria, nuclear positioning, etc.) during the three last stages of zygote germination in the *Allomyces:* uniform (isometric) state monopolar rhizoid (rh) emergence bipolar stage with hyphal (h) outgrowth. After Turian, 1969.

(C) Unequal and polar cell divisions: (I) in pollen grain (A) gradient of dots (ribosomes?) Grant's C type; (B) unequal division producing a vegetative cell (v) and a generative cell (g); (C) further division of generative nucleus; (D) unusual septation in the direction of the polar gradient producing no differentiation. (II) Differentiation of the monocotyledonous type of root hairs formation: (A) gradient of protoplasmic density; (B) trichoblasts generative of unequal division; (C) developing root hairs; (D) division of trichoblasts in the direction of polarity gradient. (III) Monocotyledonous type of stomata formation, similar to that of root hairs. (IV) Unequal divisions in the leaf cells of *Sphagnum* with dots again showing a protoplasmic gradient. After Bünning, 1952.

(D) Polarity as basis of differentiation examplified by: (I) stages (A-C) of division of an apical *Chara* cell producing two cells with different contents (B). (II) Stages (A-C) of division of an apical *Dictyota* cell in the direction of the axis of polarity, thereby producing two equal, undifferentiated cells. After Bünning, 1952.

(E) Light (L)-induced structural orientation polarity in the germinating spore of *Equisetum:* (I) unpolarized spore with central nucleus (N) and uniformly distributed chloroplasts (chl); (II) early polarization by changed positions of plastids and nucleus; (III) first nuclear division followed by (IV) asymmetric septation(s) preceding rhizoid outgrowth from the colorless basal compartment. After Nienburg, 1924, adapted from Sinnott, 1960 (I-III) and Grant, 1978 (IV).

which hardens to form a close-fitting, cellulose-like wall (Wardlaw, 1952). This quantitative change — still growth — is followed by the first qualitative change — differentiation — which is the appearance at one peripheral point of a rhizoidal outgrowth. The first cell division then takes place in the plane at right angles to the emerging rhizoid (Fig. 20 A). This highly asymmetric division is preceded by development of polarity in the egg, with the consequence that each daughter nucleus is delivered from the division into a different cytoplasmic environment; this in itself will tend to fix and accentuate the polarity. The division of the single egg cell will therefore give rise to daughter cells with different potentialities, one delimiting the rhizoid, the other developing into the frond or green thallus. The rhizoidal area is characterized by the intracellular accumulation of mitochondria, Golgi and associated vesicles, as well as the localization of sulfated polysaccharides in the cell wall. Quatrano (1978) has used the sulfated polysaccharide fucoidin as a localized, cell specific product to study the events required for its incorporation into the cell wall at a site determined by an extracellular gradient.

The initial generation of polarity in an initially *non*polar cell such as the *Fucus* egg is provoked by many types of external factors (pH, light, etc.). In the absence of any external stimulus the polarized outgrowth takes place at a random location on the surface of the cell, but with a substantial delay. According to Meinhardt's model (1984), "there is a competition between each surface element of the cell and one area will eventually win. The smaller the externally-imposed asymmetry is, the lower it takes for one part of the cell to dominate".

On the assumption that it was not already present in the unfertilized egg, polarity of the developing germ tube is determined by the factors which determine the position of the rhizoid. Colchicine does not block axis fixation or photopolarization, but prevents the first oriented cell division (perpendicular to the rhizoid axis). Therefore, some cytoskeletal basis other than microtubules and the spindle apparatus is responsible for the original polar axis. It follows that the plane of the first cell division is predetermined by the previously set rhizoid axis. This polar axis fixation and the localized accumulation of macromolecules can be experimentally directed by light. In an attempt to separate these events in time from each other, zygotes exposed to unilateral light were treated with reversible inhibitors of rhizoid formation. When the source of orienting light was rotated after untreated controls had established a fixed axis, rhizoids formed from the shaded side of the first orienting light, indicating that a fixed axis was established in the presence of the rhizoid inhibitor. Cycloheximide and sucrose-treated zygotes responded in this manner i.e. these inhibitors uncoupled fixation from localization. Oppositely, cultures treated with cytochalasin B (CB) responded to the second orienting light indicating that this inhibitor prevented axis fixation. However, also CB prevents axis establishment and axis fixation by light, it does not disrupt the orientation of a previously induced axis (Quatrano, 1973). Moreover, it was experimentally shown (Quatrano, 1978) that the prevention of axis

fixation by CB results in the lack of fucoidin localization due to an apparent disruption of the directed transport of Golgi and associated vesicles.

What other localized events occur at the presumptive site of rhizoid formation before and during axis fixation, and what is the event blocked by CB? The endogenous electric currents associated with cell polarization are disrupted suggesting that a pattern of actin microfilaments may transport Golgi-derived vesicles containing calcium channels selectively to the growing tip (Brawley and Robinson, 1985).

Nuccitelli (1978) has shown that the influx of ions and secretion of wall material occur at the presumptive rhizoid site *before* fixation of a light-induced polar axis. *Pelvetia fastigiata* zygotes exhibit both an accumulation of cytoplasmic vesicles and a clear area between the plasma membrane and cell wall at the rhizoid pole several hours prior to rhizoid emergence. This "cortical clearing" is most likely material for cell wall formation accumulating intracellularly and being secreted. In addition to cortical clearing, local fluxes of inward directing ions clearly precede rhizoid formation in *Pelvetia,* and can be localized in the plasma membrane regions of the presumptive rhizoid site before axis fixation. An ultrasensitive vibrating probe (Jaffe and Nuccitelli, 1977) was utilized to detect currents entering and leaving various surface regions. Using the probe, Nuccitelli (1978) demonstrated that shortly after fertilization the spatial current pattern around the *Pelvetia* zygote is shifted between several inward current regions. However, as the time of fixation approaches, these inward currents were concentrated at the presumptive site of rhizoid formation.

As summarized by Quatrano (1978), the time-course of events within the algal zygote could be the following: 1) The detection of inward direction current in the plasma membrane following fertilization. 2) Inward currents directed toward the presumptive site of rhizoid formation by an external gradient e.g. light; this represents the first sign of localization. 3) Accumulation and secretion of vesicles containing cell wall material (cortical clearing). 4) A process sensitive to cytochalasin B and insensitive to cytochalasin H and sucrose which fixes this site of intracellular ion accumulation and vesicles secretion for subsequent localization of particles and organelles needed for wall extension. 5) The incorporation of the acidic polysaccharide fucoidin and other polysaccharidic materials into the rhizoid wall.

b) *Stalks (rhizoid-cap poles)*

The inherent polarity of cells of algal threads of the fresh-water *Cladophora glomerata* was demonstrated by regenerative behaviour following temporary plasmolysis in 20% salt solution (Czaja, 1930). Deplasmolyzed cells began to enlarge by breaking out of their wall, and proceeded to regenerate new filaments; then, at the basal end of each cell, a new rhizoid was formed and from the apical end, a new thallus. Reversal of polarity in the threads of *Cladophora* was produced by centrifugation, showing again the close relation between the distribution of materials

in the cytoplasm and the polarity of cells. Similarly, in bits of tissue cut from *Enteromorpha,* the cells near the apical portion of the piece regenerated papilla-like structures while the cells at the base formed rhizoids (Müller-Stoll, 1952).

A case where the cell obviously has a bipolar character is the marine green alga *Acetabularia* with its cap at one end and its rhizoid at the other (Fig. 22). A fragment derived from the center of the cell will regenerate both basal and apical ends (Hämmerling, 1955). Much speculation has resulted from experiments with *Acetabularia mediterranea* and *A. Wettsteinii.* The thallus of this plant, described as uninucleate in the vegetative state, shows considerable differentiation, consisting of a basal rhizoidal portion with the nucleus, a stalk with sterile whorls or hairs, and an umbrella-shaped apical part. That the nuclear products are specific is concluded from formative effects obtained by grafting a piece of stalk of *Acetabularia mediterranea* on a rhizoid containing the nucleus of *Acetabularia wettsteinii.* Development of the scion then assumed the character of the stalk. It was then concluded that the polarity and formative effects were not due to an intimate structure of the cytoplasm nor to a metabolic gradient. The mechanism which controls the flow of the substances and their formative effects in the cytoplasm remains unexplained.

Cap differentiation in *Acetabularia* depends upon the release of "morphogenetic substances" (believed to be RNA) from the nucleus but it does not require the presence of this organelle for expression. Anucleated stalks can form the species-specific cap as well as regulate the levels of certain enzymes, suggesting that long-lived or stable mRNA is implicated in the genetic control of this localized differentiation (Quatrano, 1978).

4. *Cryptogamic spores (rhizoid-thallic poles)*

Events at cryptogamic spore germination illustrate well Bünning's hypothesis (1957) stating that there is polar cytoplasmic differentiation within the mother cell. A few important questions still remain about the operation of this hypothetical scheme for the induction of cellular differentiation: what is the origin of polarity in the mother cell? Why is geometrical asymmetry of cytokinesis an obligate step in so many instances?

From a swelling moss spore can emerge a primary rhizoid and/or a chloronema (Heitz, 1940). The young outgrown chloronema pushes out on the side of the spore toward the light, and the rhizoid forms at the opposite end, indicating that here, as in the *Fucus* egg, its polarity is determined by light. In several moss species, Fitting (1949) was able to reverse this polarity by reversing the direction of the light. In this way the young protonema becomes converted into a rhizoid. The tip of moss protonema responds phototropically and polarotropically, i.e. it perceives the vibration plane of the light. The polarotropic orientation of primary protonemal

growth of *Physcomitrella* depends on the wave length and the photon flux rate of monochromatic light, and the cell elongates parallely to the electrical vector of plane-polarized light in blue light (Schnepf, 1986).

The clear dorsoventrality expressed by the division of the apical cell in leafy liverworts must be regulated by external factors. Gravity is probably an important source of positional information in the bipolar gemmae of *Marchantia polymorpha* which possess two identical apical cells on each side. Gravity determines which will divide to produce the double dorsoventral structure of the outgrowing thallus (Halbsguth, 1953).

The moss protonema has been considered as an hormonally-controlled "morphogenetic system" in which an electric current was measured entering the tip of each growing region, namely the main filaments and side branches (Bopp, 1980). In an electric field the spores of *Funaria* tend to form the primary rhizoids toward the positive electrode. This response appears to be mediated by active calcium ion uptake as suggested by the predominant formation of those elongating structures towards the side of highest concentration in the Ca^{2+}-ionophore A 23187 (Chen and Jaffe, 1979). By contrast, subsequent growth of both rhizoids and chloronemata was directed towards the negative electrode. Other environmental orienting effects have been described by Schnepf (1982).

The spore of the horsetail *Equisetum* shows no predetermined, external or internal polarity and its germination is followed by division into two unequal cells. Light was shown to be the directing factor in the division of this apolar spore (Stahl, 1885). A redistribution of cytoplasmic material first takes place, especially an aggregation of chloroplasts on the illuminated side (Nienburg, 1924). Then only occurs the alignment of the nuclear spindle parallel to the direction of light (Fig. 26 E) followed by unequal division of the cell by a wall laid down at right angles to the gradient of light absorption. The axis of the division figure and the daughter nuclei become arranged in such a way that the more strongly illuminated daughter cell becomes the primary prothallial cell, and the one on the darker side, the rhizoid cell. The point of emergence is highly basophilic and numerous mitochondria appear in differentiating rhizoids (Nakazawa, 1956-60). The first, larger cell is chloroplast-rich and will later give rise to the plant shoot, the second smaller cell is chloroplast-poor and will give rise to the root-like rhizoids. The spores of *Equisetum* require only a few seconds of irradiation to induce localized germination several hours later (Haupt, 1957). The very short duration of light action and its low intensity necessary to give rise to a certain spatial development in the cell sometime later lead therefore to conclude that this morphogenetically effective physical factor functions only as signal and does not supply the cell with the necessary energy for the subsequent reactions (Weisenseel and Kicherer, 1981). Preceding stable polarization of intrasporal content, there has been observed a period of labile polarization when the orienting effect of illumination could still be negated by subsequent illumination from the opposite

direction (Mosebach, 1943). The spores of *Equisetum* respond to applied electric fields as the zygotes of *Fucus* and *Pelvetia* by germinating in a parallel direction to the field (Bentrup, 1968).

According to Miller and Bassel (1980), "polarity may exist in the fern mother cell as a *pre*existing, persistant state, or it may arise *epi*genetically in the mother cell which initially is non-polar". As a first experimental answer, these authors found that in most caffeine-treated spores, the nucleus moved back to the center of the spore in which cell wall formation and rhizoid differentiation were both blocked. However, there were infrequent cases of caffeine-treated spores in which a feature characteristic of rhizoids was expressed, in the absence of asymmetric cell division. The explanatory model proposes that spores have a *pre*-existing stable polarity, such that one end of the spore is differentiated. Previous observation that the direction of outgrowth of the protonema and rhizoid could not be influenced by either unilateral light or plane-polarized light also supports this model (Miller and Greany, 1976).

5. *Higher plant cells*

a) *Eggs*

In higher plant, the zygote or fertilized egg is a highly vacuolated cell suspended in the embryo sac. An initial asymmetric division of the egg defines the polarity which persists throughout the early embryogenesis. The smallest cell, with dense content divides further into the embryo while the larger, vacuolate cell gives rise to the suspensor (Burgess, 1985).

b) *Epidermal cells*

Differentiation of cells in many plant tissues is apparently linked to an *asymmetric* — or unequal — proliferative cell division in the proximal region of the meristem (Barlow, 1984). There are two examples in which the differentiative stimulus is related to the cell's position: trichosclereids which are hair-like cells develop in the cortex of aerial roots such as those of *Monstera*; epidermal root hairs (for their growth, see VI.A.2g). In case of the trichosclereid initials, commitment after signalization may consist of some sort of cytoplasmic polarization, since in many instances the small daughter cell has the more densely-staining (basophily?) cytoplasm. Thus, "differences between the time of commitment and the onset of differentiation can lead to patterned development which may grow if the products of the differential division continue to divide and maintain there the differentiated state" (Barlow, 1984). It is this process which leads to the files of hairs, interspersed by files of hairless cells (Fig. 26 C; Bloch, 1947; Bünning, 1952).

In the leaves of the moss *Sphagnum,* stomata and root hair initials (trichoblasts) as well as chlorophyll-bearing cells can originate by a polar separation of cells with denser protoplasm, probably resulting from a synthesis of new protoplasm (Bünning, 1952). In moss leaves, the apical cell is the first to cease to divide, and it starts to differentiate. This differentiation is associated with cell elongation and the final cell-net depends on the relative amount of elongation of different longitudinal and transverse cell walls (Bopp, 1984). The regular change of division planes results in the equal distribution of daughter cells to either side of the apical cell, forming two symmetrical halves. The descendants of the apical cells produce typical strips of cells (Sych, 1982). In the production of the different types of cells, unequal, asymmetric cell division must be involved and was clearly demonstrated in the leaves of *Sphagnum*. Positional information is necessary for this division pattern as expressed by the postulated gradient of a still unknown substance (Bopp, 1984).

The glandular hair or trichome arises on leaves of *Callitriche* as a polarized extension of one epidermal cell. The extended cell then divides asymmetrically to give rise to a basal cell and a stalk. This raised cell further divides into the multicellular head of the glandular trichome (Burgess, 1985).

The stomatal complex also develops from epidermal cells (Fig. 26 C). Prior to the initiative division, the nucleus of an epidermal cell migrates to an end to give a polarized structure. The asymmetric division gives rise to a small guard mother cell and to a second daughter, vacuolate cell. In its turn this last cell becomes polarized in the same sense as the original epidermal cell; it then divides very asymmetrically. The final developmental stage of the complex is marked by a symmetrical division of the guard mother cell, giving rise to two similar guard cells surrounding the stomatal pore (Bünning, 1957; Burgess, 1985).

In the guard cells of *Allium,* colchicine severely disrupts both the localization of wall deposition and the orientation of new cellulose microfibrils while inhibitors of actin-myosin based microfilament systems do not interfere with normal microfibril orientation in these cells (Palevitz, 1982). Microtubules thus appear to be the major contributors to the microfibrillar orientation in the stomatal complex.

6. *Higher animal cells*

a) *Eggs (animal-vegetal poles)*

If the genome of a differentiating cell varies only in its expression and not in its information content, then we must consider what methods are used by the organism to establish cytoplasmic differences in the cells of the embryo. Apparently this is accomplished by lying down chemically different regions of the egg during oogenesis and having certain regions segregated into specific cells by early cleavage.

The cytoplasm itself of the unfertilized egg is often not homogeneous. Most animal eggs contain stored food material or yolk which, since it is usually concen-

trated in one part of the cell, establishes a distinction between animal and vegetal hemispheres, i.e. it brings about a primary polarization of the cell along the so-called animal-vegetal axis: the *animal* pole is located by the side of polar body formation, while the *vegetal* pole is marked by yolk accumulation settled opposite the egg nucleus.

Mosaic eggs (a^2, etc.) show regional cytoplasmic differences which are so marked that the embryos they produce cannot compensate for missing portions of cytoplasmic materials. *Regulative* eggs (a^5, etc.) give rise to daughter cells capable of developing to complete embryos; the early embryos can regulate their development to compensate for missing portions of cytoplasmic materials. However, both kinds of eggs are difficult to separate (a^1).

A primary polar axiation must be established as an expression of some regional difference(s) in the egg as unravelled by the classical centrifugation experiments of Morgan and Spooner (1909). Their conclusion that the organization responsible for polarity occurs in the stiff outer egg cytoplasm (cortex) was confirmed by the grafting experiments of Dalcq and Pasteels (1937) who proposed the existence of a cortical field. "Morphogenetic substances" are therefore localized in the egg cortex and these substances are probably responsible for the early patterns of differentiation.

In eggs with large amounts of dense yolk such as those of amphibians, the axis of egg polarity approximates the axis of gravity, and the egg floats with its animal pole upward. In eggs with relatively little yolk, such as the sea urchin, the yolk is evenly distributed and the manner in which the egg lies is unrelated to its animal-vegetal axis.

Since polarity is generally not evident in the very early stages of oogenesis, i.e., in the oogonia, it is believed to arise during the growth and differentiation of the oocyte, being imposed on the unpolarized germ cell from the outside (Karp and Berrill, 1981). In oocytes of echinoderms and molluscs, there is a predictable relationship between the position of the oocyte within the ovary and the future animal-vegetal axis. The point at which the oocyte is attached to the ovarian wall, and therefore the point of entrance of supplies to the oocyte from the outside, becomes the vegetal pole. By contrast, in mammals oocytes are completely surrounded by ovarian follicle cells, and there is no obvious relation of any ovarian feature to polarity. As in plants, when an animal egg cell divides, its asymmetrically spread cytoplasmic materials restricted to one or the other pole will be distributed unevenly between the two daughter cells (unequal division), depending on the orientation of the plane of cleavage.

a^1 *Echinoderms (sea urchins)*

The simple but powerful experiments of Morgan and Spooner (1909) on these centrifuged eggs demonstrated that even "regulative" embryos begin development

with an axis of polarity, at least, preformed. The sea urchin egg is therefore *not* isotropic and this leads to think that there are *not* two perfectly separable and distinct kinds of development, "mosaic" and "regulative" (Jeffery and Raff, 1983).

In his monumental text-book, E.B. Wilson (1925) had emphasized that the "morphogenetic determinants" may not be in their final locations at the very start of development. It is by following the fate of a natural egg marker such as the red pigment band in *Paracentrotus lividus* (Morgan, 1927) that it has been possible to know that defined regions of the egg cytoplasm can give rise to particular structures during the course of embryogenesis. Since, architecturally-differentiated domains of the egg cytoplasm have been evidenced by differential organization of the molecular cytoskeleton that match the domains of those morphogenetic "plasms". If one could demonstrate a maternal mRNA localized in the cytoplasm, the specificity for such a localization must lie in the cytoskeleton as well as in the mRNA (Raff, 1983).

a^2 *Molluscs and Worms (Annelids and Nematodes)*

In molluscs and annelid worms, the normal first cleavage partitions critical cytoplasmic constituents asymmetrically and is therefore a *determinate* cleavage; when the daughter cells are separated, they do not have equivalent developmental potentialities. In some molluscs (mussels and sea snails), a protuberance called the polar lobe develops on the fertilized egg cell just before the first cleavage occurs. The plane of the cleavage is oriented in such a way that one of the two daughter cells receives the entire polar lobe. Something in the polar-lobe material must be essential for formation of mesoderm, and its asymmetric distribution makes the first cleavage of the egg a determinate one.

Cytoplasmic localizations are established during oogenesis in the mosaic egg of the nematode *Caenorhabditis elegans* and appear to exert fixed determinative influences after cleavage and parcelling out of cytoplasm (Hirsh, 1979). The first divisions of its zygote are also asymmetric, producing a larger cell at the anterior than at the posterior pole. Subsequent division planes are not orthogonal as in sea urchins and so the arrangement of the embryo cells is more complex (Loomis, 1986).

a^3 *Insects*

Regional differences in morphological as well as chemical properties occur in many eggs along a specific axis — the egg axis. This egg axial polarity subsequently influences development, the anterior pole region of oblong insect eggs normally differentiating to head-thorax segments and the posterior pole to abdominal segments. This *anterior-posterior* polarity, encoded by *bcd* and *nos* genes (see VIII.B.2d[1]), is laid down when the egg of *Drosophila* is formed in the ovary under the control of the maternal genome. The mutation *dicephalic (dic)* affects not only follicle development, thereby altering the antero-posterior polarity of embryonic patterning, but also

the egg shell polarity (Lohs-Schardin, 1982). Other maternal-effect mutants (*dl, snake,* see VIII.B.2d[1]) affect the *dorsoventral* polarity.

Observations of maternal-effect mutants suggest that egg cytoplasm contains substances which define the spatial coordinate of the futur embryo but little is currently known, however, about the substances coded by the maternal genes that endow the egg cytoplasm with its spatial polarity. It is only recently that several mRNAs have been isolated that are localized to either the animal or vegetal hemisphere (Rebagliati *et al.,* 1986). One of these mRNAs, Vg1, has been recently found (Yisraeli and Melton, 1988) to be distributed homogeneously throughout the cytoplasm of early-stage oocytes and localized during oogenesis at their vegetal cortex; translation of this Vg1 mRNA is not required for the localization of the message itself. Thus, «the information necessary to interpret the animal-vegetal polarity in oocytes is present in the naked mRNA transcript» (Yisraeli and Melton, 1988). These last authors make the interesting suggestion that «by specifically interfering with different cytoskeletal elements, and by identifying cytoplasmic components that specifically recognize Vg1 mRNA, it should be possible to detect the localization process and to understand *how* polarity is established and interpreted.»

Primordial germ cell differentiation, first studied in Chrysomelid beetles by Hegner in 1908, is initiated when nuclei arrive at the polar region of the oblong egg and enter a special region of cytoplasm which appears to function as the germ cell determinant (Davidson, 1986). Hegner succeeded in selectively destroying the polar cytoplasm with a hot needle before the peripheral movement of the nuclei had occurred. In the mosaic type of eggs of higher Diptera or of Lepidoptera, the main regions of the body appear to be already mapped out in the cortical plasma at the time of lying, before the zygote has even begun to divide. The entire cortical cytoplasm of the egg is rich in nucleoprotein, but the relative importance of protein and nucleic acid in the early stages of determination is uncertain (von Borstel, 1957).

Suggestively, eggs of *Formica* with a large accumulation of nucleic acids at the hind pole develop into normal females while those with a smaller accumulation become workers (Bier, 1954). In the Cecidomyid midges the germ line nuclei retain their full complement of chromosomes, whereas most of the chromosomes are lost from the future somatic nuclei. But if the somatic nuclei are exposed to the nucleoprotein of the pole plasma the elimination of chromosomes is arrested (Geyer-Duszynska, 1959). It may be that the characteristic effects, which the different regions of the cortical plasma exert upon the nuclei that enter them, depend upon differences in the nature of the RNA content of each zone.

Convincing evidence that causal pole cell determinants are localized in the posterior region of the *Drosophila* egg was provided by UV-irradiation (Geigy, 1931) and by cytoplasmic injection (Illmensee and Mahowald, (1974). The posterior polar cytoplasm can specifically induce pole cell formation and it contains substances necessary for germ cell formation. However, additional demonstrations of the ability

of ectopically induced pole cells to give rise to functional gametes is clearly required to secure the proposal that the polar cytoplasm actually determines the germ cell lineage (Davidson, 1986).

Since the data from eggs of lower insects clearly contradict the mosaic hypothesis, embryonic pattern formation in these forms required other explanations. Seidel (1961) first suggested that pattern formation ensues from a region near the middle of the prospective germ anlage, called the *differentiation center.* According to Seidel's interpretation the immigrating cleavage energid triggers in the polar ooplasm a change of physiological state which propagates anteriorly until it reaches and "activates" the differentiation center. Planning to test the relevance of Seidel's ideas for eggs from another insect group, Sander (1984) performed large series of ligation experiments on leafhopper eggs, varying both site and stage of ligation. If both fragments of the leafhopper egg continue developing after early ligation, the partial germ bands they produce do not add up to the complete pattern: some segments are formed neither in the anterior nor in the posterior fragment, and thus a gap remains somewhere in the middle of the germ band pattern. Details of the gap phenomenon suggested that interaction between posterior and anterior egg regions in the leafhopper (and most other species) is not one-way but mutual, and this leads to the assumption of the second (anterior) gradient.

Hideo Yajima (1964) showed that centrifugation or local UV-irradiation can inflict global changes which lead to "double abdomen" or "double cephalon" patterns instead of a normal larva. The analysis of ooplasmic determinants in Yajima's "anterior formative locality" was carried down to the molecular level by Kalthoff (1983) in an impressive series of experiments on double abdomen induction in chironomids (Fig. 35 C).

A good correlation has been recently found between preliminary physiochemical data and the morphologically confirmed determinants (Schwemmler, 1987): in the ooplasm of unfertilized egg, fermentation activities dominate with an optimum at the posterior pole, upon which the similarly glycolytically active endocytobionts have a direct or indirect influence; in the fertilized egg, the mitochondrial respiration activities gradually increase, beginning in the anterior pole at the cost of glycolysis, and then dominate after egg deposition.

a^4 *Ascidian-Tunicates*

Three different ooplasms, the ectoplasm, the endoplasm, and the myoplasm, are visible in living eggs and sectioned material. They each occupy defined territories in the unfertilized egg. The transparent ectoplasm, derived almost entirely from the sap that escapes from the germinal vesicle (GV) at the time of maturation, is located in the animal hemisphere. The yolky endoplasm surrounds the ectoplasm in the animal hemisphere and almost completely fills the vegetal hemisphere. The myoplasm

occupies the entire cortex of the egg and contains pigment granules which are tinted yellow in *Styela* and orange or red in *Boltenia* (Conklin, 1905).

The unfertilized *Boltenia* oocyte has three major voltage-dependent currents carried through Na^+ and Ca^{2+} channels activated by depolarization, and inwardly rectifying K^+ channels, activated at potentials negative to rest. Electrophysiological changes then occur between fertilization and the eight-cell stage. Lineage specific development of calcium currents have been measured by the whole-cell patch clamp technique during ascidian embryogenesis (Simoncini *et al.*, 1988).

In ascidian eggs, mRNA molecules are tenaciously associated with specific ooplasms as implied by their remarkable resistance to mixing during ooplasmic segregation (Whittaker, 1979; Jeffery, 1984).

a^5 *Amphibians*

During oogenesis, the amphibian egg becomes polarized along its animal/vegetal (A/V) axis (Fig. 36 A). That polarity can easily be seen in the superficial pigmentation pattern of virtually all species of amphibians eggs, which typically are darkly pigmented in the animal hemisphere and lightly pigmented in the vegetal hemisphere (Malacinski, 1984). The pigment polarity is complemented by a polarization of internal components: the smaller, less densely packed yolk platelets in the animal hemisphere, the larger, more densely packed platelets, in the vegetal hemisphere. Around the A/V axis, the egg is radially symmetrical, i.e., cross sections which pass through the A/V axis are identical at virtually all longitudes around the egg's equator.

To explain the origin of the animal-vegetal axis in oogenesis, early embryologists proposed that the native oocyte gains its polarity from the asymmetric environment of its follicle. Gravity was suggested to produce the yolk gradient in amphibian eggs (Pasteels, 1951). However, in general, the arrangement of the internal contents has no relation to the position of the cells in the gravitational field, as particularly shown in the *Drosophila* retinulae (Waddington, 1962). It has been more recently shown that oocytes are not aligned in the follicle relative to the stalk, and are random with respect to the frog's body axis and to the gravitational vector; therefore the notion of the environmental induction of polarity has lost support (Jeffery and Raff, 1983). The alternate idea is that animal-vegetal polarity of the egg originates from prior internal structure such as the nucleus-centrosome axis of the oogonium or earliest oocyte.

As for the difference in pigmentation of the animal-vegetal, dark-light hemispheres respectively, it has been localized in the cortex of the ovocytes in which pigment granules are first uniformly distributed and only later restricted to the dark hemisphere. An asymmetric location of the Golgi system might explain such differential pigmentation. If the Golgi terminus of the cell were a site of active plasma membrane insertion, then a new pigmentless surface might originate from one pole,

while the old and less expanding surface might gradually recede with all the pigment granules (Gerhart *et al.*, 1983; Malacinski, 1984). This proposal would provide a plausible scheme for the genesis of the specialized polarity of the egg from a set of inherent, general, polarized cell functions.

While, during oogenesis in insects (*Drosophila,* see p. 213), the initial cue for establishing the anterior patterning system may by the cellular organization of nurse cell/oocyte complex (MacDonald and Struhl, 1988), in amphibians (*Xenopus,* etc.), initial trancripts become progressively localized within the oocyte, "suggesting that mRNA localization depends on a prior localization of the presumed receptors or anchoring molecules within the egg" (Rebagliati *et al.*, 1986).

Amphibian eggs can be considered as models for A/V polarity. According to Malacinski (1984), it could best be simply defined as "the stratification from animal to vegetal pole of cytoplasmic components which is built up during oogenesis". The significance of the differential distribution of yolk platelets and other inclusions (e.g. germplasm) should, however, not be overemphasized because, even after the cytoplasm organization is perturbed by egg inversion, substantial pattern regulation can ensue. As for the function of that polarity, it could be "for early pattern formation (e.g. cleavage and involution), perhaps simply to ensure a high frequency of orderly development following egg activation". For later pattern specification, A/V polarity could be most significant for primary embryonic induction, that is, for insuring that cells in the animal hemisphere develop the capacity to respond to the action of the primary embryonic organizer (see VIII.B.2f).

A correlation appears to exist between a double gradient of metabolism and the double gradient of differentiation. The causal link between these two phenomena comes from the analysis of the metabolic gradient under conditions that are known to affect the morphogenetic gradients. The two appear to be linked. For example, lithium treatment abolishes the center of metabolic activity at the animal pole without affecting that of the vegetal region. Animalizing agents have the opposite effect and leave the embryo in possession of an intact animal gradient but lacking a counterbalance from the opposite pole. In each case, the ratios of the two influences are greatly disturbed, and the expected abnormal differentiation results.

a[6] *Fishes, Reptiles and Birds*

Yolk platelets take up almost the volume of most of these eggs. Cytoplasm is therefore limited to a relative cap or germinal disk on the top of the yolk. The first two cleavage furrows are orthogonal, but division is incomplete namely the cells are open to the yolk at the bottom (Fig. 8.4. in Loomis, 1986).

a[7] *Mammals*

Their eggs are small with much reduced yolk content. Polar bodies are formed before the first cleavage (Loomis, 1986).

Spermatozoons born from spermatids by the process called spermiogenesis are beautifully constructed polar cells, with their two-part head (nucleus and acid acrosome) and the mitochondria in the midpiece supplying the ATP required for the motility of the back tail (Karp and Berrill, 1981).

Early mouse embryogenesis is not only of the regulatory but also of the mosaic type. The process of differential inheritance is of crucial importance in generating cell diversity in the early mouse embryo. According to Johnson and Pratt (1983), both induction of polarity and the polarizing response are observed at the normal developmental time in embryos placed in an effective dose of α-amanitin at the late two-cell stage even though cleavage is blocked. Thus, any mRNAs required for the events of polarization must be present by this time. Whether these mRNAs are maternal or newly transcribed and embryonic is still unknown.

From oocyte to early eight-cell stage there is *pre*polar organization of membrane and subcortical cytoskeleton which is altered in a reversible and contact-dependent manner. By the eight-cell stage, the epithelial axis is established and this organizing activity is expressed by its stabilizing effect on the cytoskeleton evidenced by the apical polarization of the actin and tubulin cytoskeletons that in turn mobilize cell organelles (Fleming and Johnson, 1988).

b) *Epithelia (apical-basolateral poles)*

The ability of epithelial membranes to function vectorially depends on the polarity of the epithelial cells, observable both in the asymmetric distribution of organelles in the cytoplasm and in the segregation of different sets of proteins into two opposite plasma membrane domains, apical (luminal) and basolateral, separated by tight junctions (Sabatini *et al.*, 1983). The basal surface of the basolateral membrane is that which faces the blood supply on the serosal side, while the apical surface faces the luminal side (Fig. 27).

Epithelial cell lines can retain in culture polarity properties of natural epithelia and confluent monolayers of canine kidney cells become morphologically and functionally polarized. Like natural epithelia, canine kidney cells generate two domains in the plasma membrane, apical and basolateral, separated by tight junctions. Tight junctions are thought to be essential for maintaining the functional polarization of epithelial cells by preventing the intermixing of plasma membrane components, which must be segregated exclusively to one or the other cell surface domains. Tight junction associated-proteins have been recently isolated and cingulin characterized as an acidic highly elongated, peripheral component (Citi *et al.*, 1988).

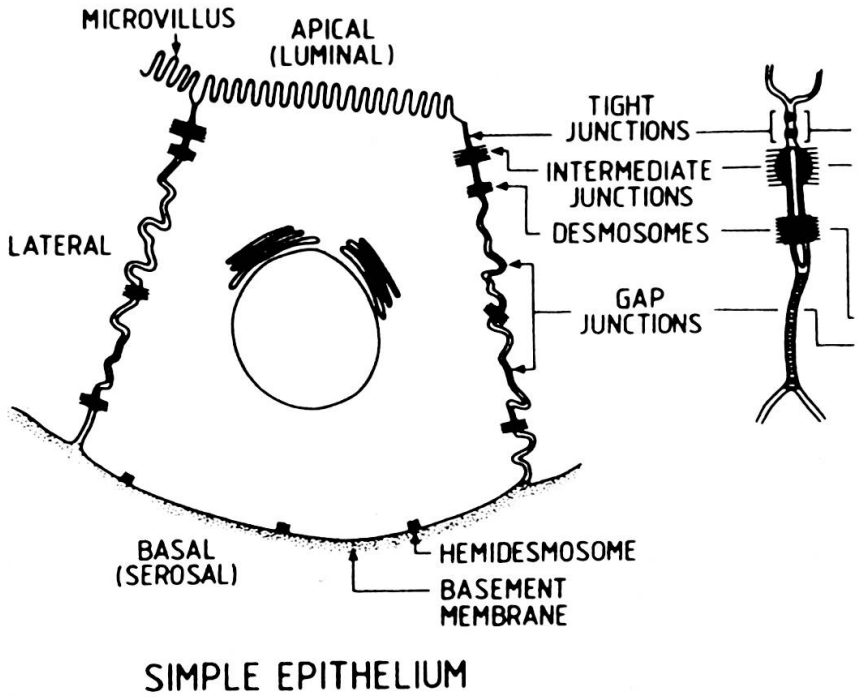


FIG. 27.

Working model depicting the organization and major proteins of the brush border cytoskeleton of a simple epithelium: apical (luminal)— basal (serosal) polarity. From M. S. Mooseker, 1985. Organization, chemistry, and assembly of the cytoskeletal apparatus of the intestinal brush border. Ann. Rev. Cell Biol. 1: 209-241, with authorization from Annual Reviews Inc., Palo Alto, U.S.A.

The two surfaces of apical and basolateral domains are characterized by markedly different protein compositions, reflecting the ability of the cell to target newly synthesized membrane proteins to specific regions of the cell surface (Simons and Fuller, 1985; Matlin, 1986). This targeting capability is also apparent in the polarized release of secretory products, distinct sets of secretory proteins being released from their apical and basolateral poles (Kondor-Koch *et al.*, 1985; Gottlieb *et al.*, 1986; Gonzalez *et al.*, 1987; Roth *et al.*, 1987). The fundamental characteristics that allow this vectorial transport across an epithelial cell are the differential sorting and insertion of transport proteins either in apical or basolateral plasma membrane, and the preferential association of endocytosis and exocytosis with one or the other pole of the cell. That plasma membrane proteins are asymmetrically distributed between the apical and basolateral surface is additionally demonstrated by the asymmetric budding of enveloped viruses from only one plasma membrane domain (Rodriguez-Boulan and Pendergast, 1980).

According to Caplan *et al.* (1987), secretion of basement membrane components (heparan sulphate proteoglycan (HSPG) and laminin) takes place from the basolateral cell surface and this polarized release results from active sorting. The

sorting process which mediates this polarized secretion requires an acidic intracellular compartment. MDCK cells treated with NH_4Cl to raise the pH of their intracellular compartments secrete laminin and HSPG by a default pathway which leads to their release in roughly equal quantities into the medium of both the apical and basolateral compartments. The nature of this acidic compartment and its role in sorting remain unknown. It is tempting to postulate that the sorting of basolateral secretory proteins (that is basement membrane components) occurs by mechanisms similar to those that mediate diversion of newly synthesized lysosomal enzymes to lysosomes (Caplan *et al.*, 1987).

Polarization of plasma membrane domains is also an essential feature of secretory epithelial cells from exocrine glands. The surface of exocrine cells (a typical example is the acinar cell of the pancreas) is separated into an apical domain, where secretion occurs by exocytosis, and a basolateral domain, which senses variations of the internal milieu and is enriched with receptors for various hormones and secretagogues. It is unknown, for example, whether hormone release occurs through the same region of the cell surface that receives exogenous stimuli or whether endocrine cells possess distinct "sensitive" and "effector" poles. The data presented by Lombardi *et al.* (1985) clearly demonstrate polarization of plasma membrane domains in cultured pancreatic endocrine cells and provide the basis of future efforts to unmask plasma membrane polarization in endocrine cells *in vivo*. In this respect, it is worth mentioning that recent physiological data are consistent with the possibility that distinct venous (receptor-rich) and arterial (secretory) poles exist in pancreatic insulin-producing cells.

Vectorial solute transport by epithelia requires the polarized insertion of transport proteins into apical or basolateral plasmalemmal domains. In the specialized intercalated cells of the kidney collecting duct, the selective placement of an apical plasma membrane proton-pumping ATPase (H^+-ATPase) and of a basolateral membrane anion-exchange protein results in transepithelial proton secretion. Recently, it was proposed that intercalated cells can reverse their direction of proton secretion under different acid-base conditions by redirecting proton pumps from apical to basolateral membranes, and anion exchangers from basolateral to apical membranes (Schwartz *et al.*, 1985). But others have found that antibodies raised against the red cell anion exchange protein only labelled intercalated cells at the basolateral plasma membrane, providing evidence against the model of polarity reversal. Brown *et al.* (1988) have found that some cortical collecting duct intercalated cells have apical plasma membrane proton pumps, whereas others have basolateral pumps. This is the first direct demonstration of neighbouring epithelial cells maintaining opposite polarities of a transport protein. Thus, either subtle structural differences exist between proton pumps located at opposite poles of the cells, or factors other than protein sequence determine the polarity of H^+-ATPase insertion.

The interaction between membrane proteins and cytoplasmic structural proteins is thought to be one mechanism for maintaining the spatial order of proteins within functional domains on the plasma membrane (Singer, 1974). In polarized epithelial cells homologues of ankyrin and spectrin (fodrin) are localized in specific membrane domains. Nelson and Veshnock (1987) showed that ankyrin binds to the ubiquitous $(Na^+ + K^+)$ATPase, which has an asymmetrical distribution in polarized cells (Hootman, 1986). Changes in the cellular organization of fodrin result in the formation of a highly insoluble, relatively dense and stable layer of that structural protein which appears to be localized to the cell periphery and predominantly in the region of the basolateral plasma membrane of canine kidney epithelial cells in continuous monolayers (Nelson and Veshnock, 1986). The formation of this structure coincides temporally and spatially with extensive cell-cell contact, and with the development of the polarized distribution of the Na^+, K^+-ATPase, a marker protein of the basolateral plasma membrane.

The cylindrical hair cells of the inner ear are also apico-basally differentiated cells of epithelial origin (Roberts *et al.*, 1988). They are receptors which act as extremely sensitive mechano-electric transducers; they convert a mechanical force, the stimulus applied to the hair bundle, into an electric signal, the message relayed to the brain. The hair bundle is arranged with a plane of symmetry and all but one (kinocilium) of its extensions are called stereocilia. In the cell itself, structural polarity is evidenced by the exclusion of mitochondria from the apical, cuticular plate zone and the afferent nerve ending at the basal pole surface. Both the apical and the basal surfaces of the hair cell act as capacitors thin plates separating electric charges with opposite signs and both areas have channels for ions. In its directional sensitivity, the hair cell is capable of resolving any stimulus into two components: a first, along the axis of bilateral symmetry, to which the cell responds with a depolarization (one directional push) or a hyperpolarization (push in the opposite direction). To the second component, along the perpendicular axis, there is no response.

VIII. MORPHOGENETIC POLARIZATIONS

As a third aspect of development, morphogenesis is related to the multicellular level of organization and involves cellular interactions as opposed to differentiative processes occurring primarily within individual cells. Strictly speaking, morphogenesis therefore refers to the development of the shape and specific form of the organism and its individual parts. As such, it is largely a problem of coordinated movements of cells toward their specific position in the organisms being constructed. On the framework of a pattern of genotypically and phenotypically-controlled polarities, these oriented displacements of cells lead to the organismic morphogenesis in the bewildering array of living forms. All such marvellous formative achievements require the concourse of polarity at all levels of integration.

A. Plants

Polar patterns are very frequent in plant morphogenesis especially in respect to the formation of polar cells (Wardlaw, 1952; Sinnott, 1960). Physical factors, i.e. internal and surface forces are implicated in the patterning of tissues if we consider with D'Arcy Thompson (1942) that division is a mean of restoring equilibrium within an enlarging cell. To explain the polar tissue patterns, the Warren Wilsons (1984) developed an hypothesis that integrates the surface effect and the homeogenetic induction effect in terms of opposed gradients of two morphogens — auxin and sucrose — which have *sources* and *sinks* sufficiently defined to permit a simple simulation of the processes of positional control.

In most cases, the polarity of a cell is established parallely to the axis of cell elongation, and cell division usually occurs in the plane perpendicular to that axis of polarity. The determination of the anchor points of the spindle apparatus is, of course, another important pattern-formation event within a dividing cell. A high activator concentration could initiate the self-assembly of the microtubules and so determine the plane of cell division, even in an initially more or less symmetric cell. Asymmetric cell divisions, which are fairly frequent in plant cells (VII.C), could be explained in this way since the new cell wall may occur closer to the activated site. Of course, a second center of microtubule self-assembly has to be created at the opposite side of the cell and both centers thus generate a *bi*polar field by mechanisms of long-range activation and short-range exclusion (Meinhardt, 1984; Burgess, 1985).

Prat (1948 and 1951), and then Bünning (1953), dealt with the origin of plant patterns in terms of stimulatory or inhibitory fields or gradients around structures

which either produced morphogens or depleted the surroundings of them (Carr, 1984). Prat discussed the origin of histogenetic pattern in terms of physico-chemical gradients (redox, pH, etc.) and considered it as a direct result of the gradient or field. Since, it has been recognized that there is a difference between gradient theories and "positional information", a term coined by Wolpert (1969, 1971, 1981) — although the idea had occurred a century ago to Vöchting and Driesch — to "imply the existence of unknown mechanisms which cause cells in an embryo, an organ or a small animal to respond to the position along a developmental axis or within an aggregate of cells" (Carr, 1984). Wolpert's definition of pattern formation is "the specification of spatial differences", a specification implying that such differences are genetically determined. In plants, however, most patterns are epigenetic in origin and this may have some consequences on the straight application to plants of Wolpert's concept (see below). As first conclusion, the difference between gradient theory and positional information theory is that, "while gradients are primarily held by the latter theory to elicit a cellular or morphological response, it is the position the cell or meristem occupies in the gradient that determines which of a number of courses of differentiation it will take" (Carr, 1984). To assign a "positional value" to each cell in the developmental field would involve the establishment of diffusional gradients of some morphogen scaled so that each cell in the matrix can be considered to reside at a unique intersection. Moreover, each cell should interprete its assigned positional value in terms of an appropriate developmental protocol. "The cell is assumed to possess some form of "reference library" in which it can turn to the appropriate page, specified by its positional value, and read what it must do to complete its part of the overall developmental process" (Lintilhac, 1984).

Nevertheless, as already commented, and following Lintilhac's opinion, the concept of developmental control by means of positional information to explain the events associated with amphibian limb regeneration (VIII.B.2f) cannot be applied directly to plant systems where developmental events occur at regular intervals along the growing axis. Indeed, the extraordinary sensitivity of plants to structural and mechanical stimuli is qualitatively quite different from the positional sensitivity of animal cells, and depends on the mechanical coupling provided by shared rigid cell walls (Lintilhac, 1984). Moreover, "the word "position" is ambiguous since neighbouring cells retain a constant relationship to each other while continually changing their location relative to the apical pole". Lintilhac also differed from Wolpert's view that the genome includes a "set of instructions" for the construction of the adult. Holder attempted, in 1979, to directly apply the positional information theory to plant development. However, Lintilhac considers that Holder's statements can also be misleading "to the extent that they can be taken to mean that an understanding of developmental events will come only by considering them as results of differential gene activity". He has therefore proposed the alternative theory of structural epigenesis, considering plants as architectonic structures and attempting to answer

the question of "how do plant cells sense and respond to mechanical forces?". Plant response to hormonal diffusion gradients (Sachs, 1978) was considered as the most acceptable model of "cell patterning and segmentation patterns in embryogenesis" (Wardlaw, 1952) discussed as a basis for structural epigenesis in ontogeny.

1. *Embryonic polarity*

In thallophytes, bryophytes, pteridophytes, and seed plants, the earliest perceptible embryogenic development is the establishment of polarity. Axial or spindle-like development is found in the embryogeny of all these classes of plants (Bower, 1922). During plant embryogeny the embryo proper becomes polar, establishing the root-shoot axis, and thereby forms two very different types of apical meristems. "How is the root-shoot axis established and what are the developmental parameters involved in organizing the apical meristem?" The only information obtained has come from studies of *de novo* meristem organization in culture (McDaniel, 1984*a*).

At the earliest manifestation of growth in the spore or zygote, there is evidence of polarity and of filamentous or axial development; in all but the very simplest organisms, there is, from the onset, a distinction of apex and base (distal and proximal regions or poles). In short, both the sporeling and the very young embryo afford evidence of orderly or organized development.

In naked, fertilized or unfertilized ova, such as those of *Fucus,* there is apparently no specialized cytoplasmic organization which determines the polarity of the young embryo (see VII.C.3a) and the polar axis may be established in any direction. In another brown alga, *Laminaria,* the extruded but still attached ovum gives rise to an embryo whose axis coincides with that of the oogonium. In that case, as in other developing sporophytes, some of the orderly changes that take place during the ontogenic development may already have been determined by the cytoplasmic organization of the ovum.

Several facts suggest that polarity is not determined by structural features of the archegonium itself. In the encapsulated embryo in bryophytes and vascular plants, the establishment of polarity brings the embryo into a particular position in relation to the gametophyte plant or gametophytic tissue. As this early establishment of a polarized axis has many consequences in the subsequent embryogenic development, it is evident how essential it is to have some knowledge of the factors which may bring it about.

The polarity of the enlarging embryo is determined by inherent factors, or by factors in the environment and the first partition wall is typically laid down at right-angles to the axis of the embryo. In some embryos several successive transverse divisions take place and an elongated filament results: in others, divisions at right-angles to the first wall take place, and in this we see the inception of a tissue mass. This is what occurs in many bryophytes, in *Equisetum* and some other pteridophytes

(Burgess, 1985). In *Anthoceros,* however, the ovoid zygote is divided by a longitudinal wall (Wardlaw, 1955).

The classic species for study of development of higher plant embryo has been the shepherd's purse, *Capsella bursa-pastoris.* One of its most striking characteristics is the polarity of the mature or newly fertilized ovum, in which metabolites are, or quickly become, heterogeneously distributed. Where the ovum is enclosed, the physiological activity of the surrounding tissue is probably important in determining polarity. The basal two thirds of the egg contain a single large vacuole, while the cytoplasm and the nucleus are in the apical (i.e. chalazal) third of the cell. This polarity somewhat parallels that observed in yolky animal eggs, where the nucleus is restricted to the animal hemisphere of the egg. In the polarized zygote, the apical or distal pole becomes the principle locus of protein synthesis, growth and morphogenesis; whereas the basal or proximal pole is characterized by the accumulation of osmotically active substances, its cells become vacuolated and distended.

The zygote may be more or less equally divided, or it may be divided into a small, densely protoplasmic distal cell and a larger basal cell. This first division of the zygote is asymmetric and expresses the polarity which will remain throughout the life of the plant. The two daughter cells of the first division are different in size and developmental potential. The larger basal cell will produce the suspensor, whilst the smaller terminal cell will produce the embryo proper. In short, the initial asymmetric division of the zygote defines the polarity which persists throughout the development of the embryo. Consequently, the mature embryo is a highly polar structure with a differentiated apex, a procambium which will form the vascular system, and a protoderm which will form the epidermic layer. Subsequent development of the plant rests on the activities of the meristematic tissues. The main root-shoot axis of the embryo is located centrally within the embryo, with the shoot tip between the two cotyledons. At maturity, the root tip has a differentiated cap.

2. *Organismic polarities*

a) *Mushrooms*

Polarity of the sporophores (fruit bodies) of higher fungi (Basidiomycetes) has been discovered by Magnus in 1906 when he described the much more vigorous hyphal regeneration of the cortical hyphae on the pileus side than on the foot side of a bisected stipe of *Agaricus bisporus* or of *Coprinus stercorarius.* Similar experiments were also successfully carried out by Weir (1911) in *Coprinus* and Polyporaceae. Polarity is thus evident but it is not fixed as shown by Lohwag (1941) who could graft a segment of the pileus of *Fomes* back to the same pileus in an inverted position. Developmental polarity of the fruit body cap in the genus *Coprinus* has been further studied by Reijnders (1979) who depicted polarity of differentiation where development in some species was described as a rupthymenial (gills differentiation away from the stipe) while others were levhymenial (gills differentiating toward the stipe).

The most highly organized structures of the Basidiomycetes are the fruit bodies, but the vegetative mycelium is also capable of elaborating a range of other organs among which the sclerotia (Watkinson, 1979). Whereas the sclerotium develops a radial symmetry, the fruit body developmental sequence imposes a polarizing influence on the aggregate, and from a very early stage the shape of the developing mushroom is clearly evident (Moore, 1984).

Initiation of fruit body of *Flammulina (Collybia) velutipes* is first indicated by hyphal aggregation in a much-branched region of the dikaryotic mycelium (Williams *et al.*, 1985). Further extension and elongation resulted in a primordium with the typical form of a mushroom in miniature, which showed the basic apico-basal organization of tissues into a stipe, pileus and developing hymenium. Further expansion of the stipes of *C. cinereus* or *A. bisporus* occurs by a uniform incorporation of cell wall material along the length of the growing cells. This intercalary elongation contrasts with the polarized tip growth of mycelial hyphae (Gooday, 1983; Manachère *et al.*, 1983). The development of the intensively investigated fruit body of *Schizophyllum commune* (Aphyllophorales) is not at all like that in Agaricales. The inside of the tiny cup produced is lined with hymenium producing basidiospores. The infoldings of the margin of the fan-shaped fruit-body produce the typical split gills which often seem to radiate out from one point when growth is unilateral. The normal enlargement of the cap is thus multipolar and the mutant *cup* is restricted in hymenial expansion (Wessels *et al.*, 1985).

Physical environmental variables can interact with the polar development of mushrooms as well examplified by the stipitate *Polyporus brumalis* in which the effect of light (Fig. 28 B) and apical transpiration loss are additive (Plunkett, 1961). There are also interacting effects of light and temperature, which have been studied in *C. congregatus* by Manachère *et al.* (1983). In many *Coprinus* species, light can act as an inducer/stimulator and as an inhibitor.

Development of such a highly organized structure as the whole mushroom necessarily implies polarized regulation. Both Borriss (1934) and Gruen (1979, 1982) concluded from their work with *Agaricus* and *Coprinus,* respectively, that the gill lamellae were the origin of controlling factors for stipe elongation and cap expansion. Growth factors produced by the fruit-body cap were shown to influence, perhaps control, growth of the stipe of these mushrooms as experimentally evidenced by total removal of the cap which leads to cessation of stipe growth. When segments of cap were left attached to the stipe, the latter showed a growth curvature with the greatest extension growth being immediately beneath the remaining sector of cap. The gill lamellae could thus be considered as the origin of controlling factors for stipe elongation (Fig. 28 A) and cap expansion (Gooday, 1974).

The cap displays the greatest morphological, and consequently morphogenetic, complexity. By the time it has matured, three morphologically distinct cell types have been produced, for at maturity the hymenium consists of a pavement of highly

inflated paraphyses within which are embedded a great many basidia and a scattering of cystidia.

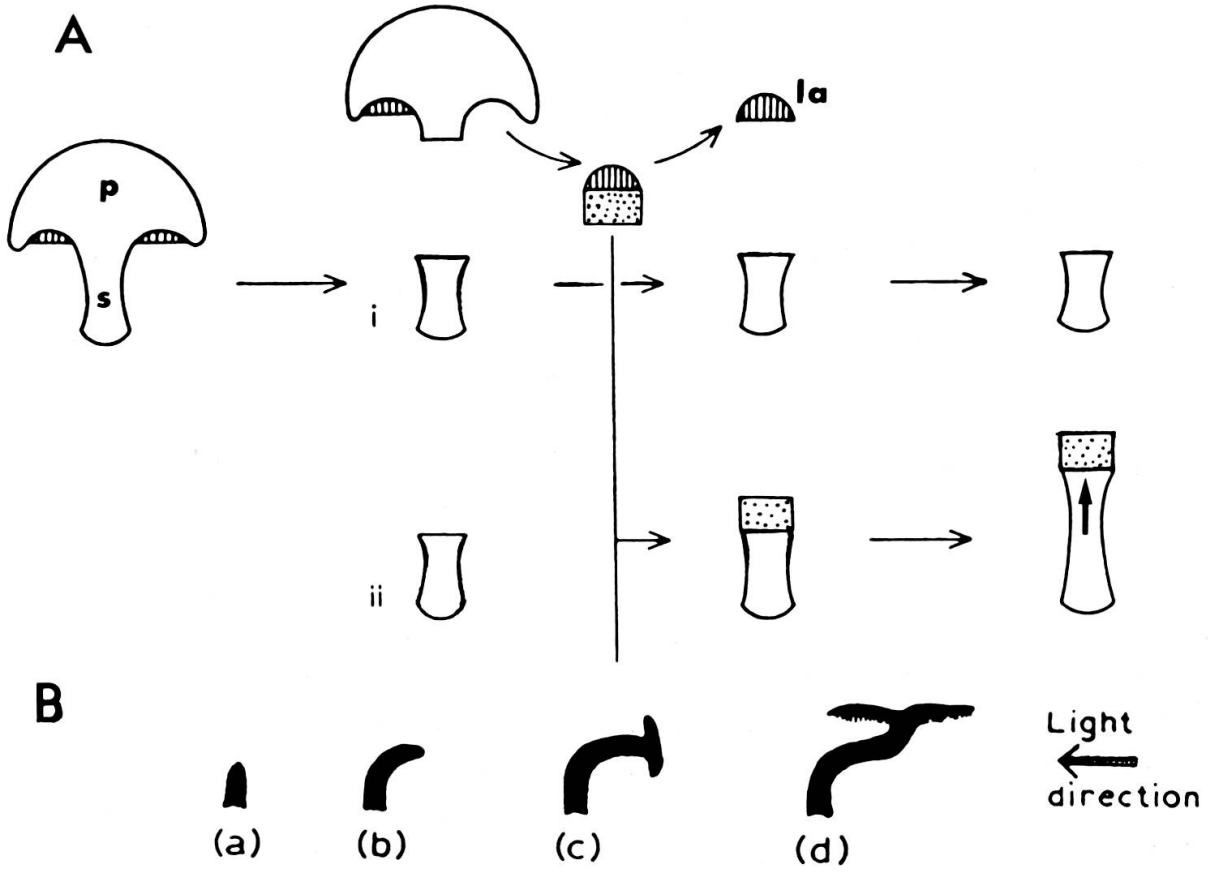


FIG. 28.

Developmental polarity in higher fungi.

(A) Hormonal stimulation of monopolar elongation growth of the basidiocarp of *Agaricus* as demonstrated with lamellae (la) substances transferred into an agar block. i = control; ii = treated elongating (arrow) stipe(s) fragment. From Gruen, 1963, in J. E. Smith and D. R. Berry, 1974, An Introduction to Biochemistry of Fungal Development. Academic Press.

(B) Response of basidiocarp of *Polyporus brumalis* to unilateral illumination: successive curvatures toward the light source (a-c) until the stipe apex is shadded by the expanding pileus when the stipe curves upward (d). After Plunkett, 1961, in Burnett, 1976.

The developmental pattern of a typical mushroom occurs along *two* axes of polarity: from base to apex, and from the inner to the outer surface. The specification of information along these two axes of polarity results in the formation of a palisade layer and thus precipitates the establishment of basic tissue domains. According to Rosin *et al.* (1985) "the concepts of positional information and polarity axes are capable of providing a basic understanding as to how the gill pattern develops, the specification of positional information being determined by the two axes of polarity". These authors then presumed that hyphal cells interprete this positional

information and differentiate into palisades which are the fundamental building blocks of the hymenium and differentiate initially into basidia and cystidia. They conclude that the differentiating agaric gill seems to be an ideal candidate for interpretation along the lines of a diffusion gradient theory as proposed by Meinhardt and Gierer (1974). These authors had illustrated "how two-dimensional patterns very similar to those observed in the agaric hymenium may be generated in response to activators and inhibitors capable of diffusing through the tissues». Rosin *et al.* (1985) further emphasized that "the gill *does* have two developmental axes to which differentiation might be referred, and the hymenial cells do become positionally differentiated in a manner apparently analogous to epidermal cell layers" (see VII.C.5b).

According to Moore (1984), "the development of these structures could provide a classic example of a morphogenetic field, the more so since the gill plate grows in two directions (inwards, towards the stipe, and along the length of the cap as the cap increases in diameter) which are essentially at right angles and which could contribute to a co-ordinating system to which cell differentiation could be referenced". Models of morphogenetic processes based on distribution of morphogens (Meinhardt and Gierer, 1974) are very dependent on adequate communications within the tissue, communications which must extend over many cell diameters. However, the developing basidiomycete hymenium is an array of separate cells in which there is no evidence for any lateral cytoplasmic contact between neighbouring cells. Recently, models involving gaseous or volatile morphogens have been proposed and founded on known metabolic events (Moore, 1981).

Pertinent as conclusions about the polar features of construction of the spore-bearing structures of mushrooms are Burnett's (1976) remarks that "basically, despite the fact that morphogenetic processes appear to transcend the limits of hyphae and apparently affect, in large structures at least, entire hyphal aggregation, their origins lie in the fact of hyphal behaviour. Growth, branching and aggregation are the fundamental processes, together with the control of direction of growth." The key to fungal morphogenesis is thus well at the monopolarly extending hyphal tip (see VI.A.2b).

b) *Colonial algae*

The green algal family of the *Volvocaceae* includes all motile colonial genera in which the cells lie in a disk or a hollow sphere. Vegetative cells are always biflagellate, surrounded by a gelatinous sheath, with a structure like that of *Chlamydomonas*. All colonies are called coenobia which exhibit a definite polarity when swimming through the water, the anterior pole of the colony always being directed forward. There is also a definite morphological anterior-posterior differentiation in size of the carotene-containing eyespots (stigma) at opposite poles of the

coenobium. The single eyespot lies at the anterior pole of a cell. Certain species have not only a progressive diminution in size of eyespots from anterior to posterior cells but eyespots may even be lacking in the lowermost tier of cells. In the most advanced genera of the series progressing from the 8-celled stage (*Gonium*), through the 32-64-celled stage (*Pandorina-Eudorina*) to the more than 1000 cells (*Volvox*), all cells toward the anterior end are vegetative and reproductive cells (gonidia) lie toward the posterior pole (Smith, 1955).

The morphogenetic inversion in *Volvox* is a gene controlled process by which algal embryos turn inside out (Sumper, 1984). By the end of cleavage, the polarity of cells in the hollow spherical embryos is the reverse of the adult orientation: the presumptive flagellar ends of the somatic cells point toward the interior of the cavity and their chloroplast ends are on the exterior surface. The major event of inversion, generation of negative curvature, "requires both microtubules-driven elongation of cells (to produce a classical "flask" shape) and cytochalasin-sensitive active migration of cytoplasmic bridges to the outermost ends of flask cells" (Viamontes *et al.*, 1979).

In the Chlorococcales, cells may be solitary or be united in non-filamentous colonies with the definite number of normally 4 cells in *Scenedesmaceae*. As a result of asymmetrical divisions, the two extreme cells bear each two teeth or spines. These algae are polymorphic and the number of cells in daughter colonies is partially dependent upon the physiological conditions of the parent cells (Trainor *et al.*, 1976).

c) *Green plants*

A vascular plant is an axially bipolar structure, since there are different apices at the opposite shoot and root tips (Sachs, 1981). This division of the plant body into shoot and root is one of the most striking aspects of plant morphogenesis and one which, in higher plants, is normally maintained with great stability throughout their life. The division of the plant body originates in the embryo with the establishment of polarity and the initiation of shoot and root apical meristems, and once established the polarity of the plant body is extremely stable and difficult, if not impossible, to reverse.

Vöchting's (1878) original evidence that the plant axis is bipolar was based on shoot-root inversion of plant axis. His finding (1892) of abnormal development of the vascular system in the inverted plant has recently led Sachs (1981) to question whether this effect of polarity on vascular differentiation is dependent on auxin and its polar transport (see c^4). Furthermore, polarity at a cellular level can also be seen microscopically in the arrangement of specialized cells in vascular strands that connect the shoots with the roots (Sachs, 1986). This observation also raised the other question of whether auxin's transport and the structural expression of polarity are related. Experiments led by Sachs answer it by showing that, when applied locally,

auxin could orient the differentiation of the new tissues and could also replace the effect of organs such as expanding leaves. Additional observations of regenerative wounded plants, further suggested to Sachs (1986) that "auxin movement and tissue polarity are linked by a positive feedback relation: the movement of auxin polarizes the tissue and this polarization orients and enhances auxin movement".

c[1] *Roots*

Moving back from the capped tip along the root axis there are three zones: the meristem zone where cells both divide and elongate; the elongation zone where cells elongate only; the mature zone where elongation ceases. The same zones are also shown by the root cap but with a proximal meristem and distal mature cells. The meristem system would be generated (e.g. in the embryo) and regulated according to such a scheme implicating the asymmetric distribution of diffusible molecules. Such asymmetry is required to account for the frequently observed asymmetric cell divisions (Burgess, 1985).

Roots as whole plants "could contain gradients of morphogens because the cells are literally awash with solutes flowing, usually in a *polarized* direction through and around them". A general proposal is that "in the growing root apex chemical signals direct the course of cellular development and that these signals vary in a quantitative and qualitative manner, according to position" (Barlow, 1984). Such signals exist as gradients, as first proposed in animal systems by Child (1941), and "it is the gradient coupled with the cell's capacity to respond, which is informational" (Barlow, 1984). Various biochemical gradients have been shown to concour to the elongation growth of roots (Cook, 1959; Pilet, 1961; Grison and Pilet, 1978).

Extension along the root is caused by turgor pressure (driving force) within the protoplast and extensibility of the wall enhanced by the acid pH detected in the zone of elongation. Auxin (IAA) is transported for the most part acropetally to this zone (Pilet *et al.*, 1979) where it may promote the extrusion of protons into the cell walls and hence render them more extensible (Rayle and Cleland, 1977). However, the excretion of H ions might not be necessarily correlated with auxin response. The direct target of the hormone action would rather be the wall matrix synthesis, thereby leading to increased epidermal extensibility (Kutschera and Briggs, 1987).

That the root had an electrical polarity was first suggested by Lund and Kenyon's (1927) pre-vibrating probe studies of surface potentials. Schrank (1945) later suggested that root-generated potential differences might affect the distribution of growth regulators. The voltage and/or specific ion gradients that result from ion current generation have then been implicated in several hypotheses of developmental controls (Jaffe, 1986). The electrical polarity of the roots just reported by Miller and Gow (1989) resembles Lund's pioneer data: current consistently enters the meristematic and elongating tissues of intact growing roots; mature non-growing root

regions are responsible for generating the outward limb of the current loop. As in several tip growing eukaryotic systems current again tends to be concentrated at the leak (i.e. the meristic and elongating tissues) rather than the pump (i.e. the non-growing mature root). Also, localized growth occurs at the sites of the leak (Jaffe, 1986). However, "the correlation demonstrated between a pattern of polarising current and root development suggests, but does not prove, a direct causal relationship between the two events " (Miller and Gow, 1989).

c^2 *Vegetative shoots*

The shoot apex is considerably more complex to describe than the root apex. This complexity arises from the wide variety of structures from which the shoot apical meristem may originate. In contrast to the root, the shoot apical meristem is superficial, and branching proceeds from the tip. The most obvious products of the growth of the shoot apical meristem are the leaves and stem of the plant. Angiosperm apical meristems are stable: there are apparently no reports of a shoot meristem transforming itself into a root meristem (Halperin, 1978).

Apical meristems in lower plants exhibit more plasticity than those of angiosperms: at the base of each leaf in *Selaginella* there are a dorsal meristem which normally forms a shoot and a ventral meristem which normally forms a root, a normal rule which can be disturbed by auxin (Wochok and Sussex, 1976; Fig. 5 in McDaniel, 1984*a*).

The phenomenon of the priority of apical over lateral growth is called *apical dominance.* One of its aspects — the correlative inhibition of lateral buds by the apical region of the shoot — is a classical example of hormonally integrated growth in plant, principally brought about by indole-3-acetic acid (IAA). In coleoptiles and roots of *Zea* the polarly transported moiety appears to be pure IAA. Because of the gradual nature of apical dominance, it is reasonable to suppose that it is controlled by gradients in the stem. The correlative signal(s) in this phenomenon and the characterizations of the exact path of transport, rate of movement and primary site of action are presently conjectural (Hillman, 1985).

Vascular differentiation as readily occurring around wounds involved the formation of strands whose polarity is at all possible angles to the original axis of the unwounded tissues (Sachs, 1984). To what extend is the reorientation of vascular differentiation influenced by the original tissue polarity axis? To answer this question, Gersani (1987) compared new vascular differentiation along the original lines of polarity in isolated tissues and at various angles to this polarity. Differentiation of vessels was found along different tissue polarities and the rate of differentiation as a function of time and IAA concentration was the fastest in the original direction of polarity.

Positional controls of shoot organogenesis involve a three-step sequence: (a) abrupt shift in polarity, (b) smoothing, and (c) bulge. The ensuing mechanism of

phyllogenesis and phyllotaxis would imply the early development of one leaf bringing on the polarity discontinuity required for the production of the next leaf. The first signs of leave initiation are periclinal divisions in a small group of cells located in one or more subsurface layers of the shoot apex. This initiation has been viewed as the consequence of preferential enlargement of cells in the direction of future outgrowth from the parental tissue. This hypothesis of polarized cell enlargement as crucial early event in plant organ initiation has been supported by experiments showing that both leaves and roots could be initiated under conditions in which concomitant cell divisions had been inhibited (Foard *et al.*, 1965; Foard, 1971).

The formation of lateral buds in leaf axils proceeds to various extents in different plants. Lateral buds may be easily visible, or they may be very much reduced. They all have in common the fact that, at some stage, their development will be arrested and will not proceed until the growth of the stem results in displacement of the bud well away from the apex, or until the apex is itself removed or physically damaged. Thus, in a typical plant stem there is a gradient not only in the age of leaves with the youngest at the top nearest the growing point, but also in the development of lateral branches. The general form of the vascular system of plant is that of a drainage system; thus a polarity can be readily assigned to all major strains. The leaves of many ferns and most seed plants, however, are supplied by complex vascular networks that can not be understood as an expression of a polar flow (Sachs, 1984). Only their abscission appears to involve a polarized Ca^{2+} regulation (Trewavas *et al.*, 1984).

c^3 *Shoot-root balance and reversal*

The shoot and root meristems may be generated and regulated according to the scheme of long-range activation and short-range exclusion. According to Meinhardt (1984), bipolar fields are generated by these mechanisms of long-range activation and short-range exclusion.

The formation of specific organs can be due to specific substances such as hormones but it may also be affected by concentration gradients of various kinds and "hence by polar (physical) as well as chemical relationship" (White, 1934). However, polar movements of growth-promoting substances such as indole-acetic acid (IAA) were detected, only high concentrations of IAA being transported acropetally (Went and White, 1939). By assuming the existence of such a polarized circulation of growth factors, Gautheret (1944) also derived interesting ideas as to how undifferentiated tissue, growing in culture, becomes organized into an axiate structure with an apical bud, leaves and roots. However, the primary cause of polarity remains still unknown, and in Gautheret's view should be sought, not at the tissue level of development, but at the cellular level.

The suggestion of Czaja (1935) that polarity is due to the movement of auxins and not the cause of it, has not met with general acceptance. The evidence rather indicates that the polar transmission of stimuli is due to the polar transport of auxins. According to Went and Thimann (1937), polarity in auxin transport is probably determined by some inherent property of the living cells: it is therefore difficult to influence it by changing the external environment. The action of growth-regulating substances, or their precursors, is closely bound up with such polarized movement in the plant.

Hormones, like other organic substances, move most rapidly in the phloem and can also be transported through parenchyma and sometimes in the xylem sap. For phloem transport of auxin and other hormones there is no evidence that direction depends on factors other than source and sink locations of the hormones and the sugars with which they are transported. For the specific transport of auxin outside the sieve tubes, the following main types of evidence point to a determined tissue polarity (Goldsmith, 1969, 1977): (a) The direction of transport for any type of tissue is fixed, independently of the presence of growing apices or any other metabolic center. Thus transport towards the roots continues not only in their absence but also when their sink activity is prevented by cold. (b) The polar effects of auxin are clearly expressed in morphogenetic events and their existence at a tissue level can be seen clearly in grafts where one of the two members was inverted. (c) Polarity is maintained even when both the source and the sink are agar blocks, whose location can readily be changed. Finally, (d) polar auxin transport (see VIII.A.2c[4]) continues against an overall concentration gradient, as shown both by measurements (van der Weij, 1934) and differentiation events, though this does not mean that such gradients have no effect on transport.

"A vascular plant can be viewed as a branched axis that grows at its tips" (Sachs, 1984). As first shown by Vöchting (1884) the very same parts of this plant axis are able to develop different apices depending on their location relative to the rest of cutting.

Although axial polarity is normally constant and irreversible (Fig. 29 A, D), in a small number of root and shoot cuttings it may be reversed by simply turning the cutting upside. Reversal seems to be easier to accomplish in seedlings than older plants (Castan, 1940). "Inversion of polarity" was obtained with etiolated pea seedling with epicotyl decapitated, inverted, and placed in water. Roots now grow out from the epicotyl and a shoot from a cotyledonary bud (Fig. 29 B).

Oppositely, the retention of polarity found to be expressed within a cutting of a stem segment of ivy could reflect gradients of hormone concentration (Castan, 1940). According to Burgess (1985) "it would be a reasonable hypothesis that the transport of auxin from the tip of a shoot to its base would give rise to an accumulation of this substance at a cut basal end, and that this would then trigger root formation. Equally, its transport away from a cut apical end would result in the formation of shoots due to a switch in the relative levels of auxin to cytokinin. Taking this view

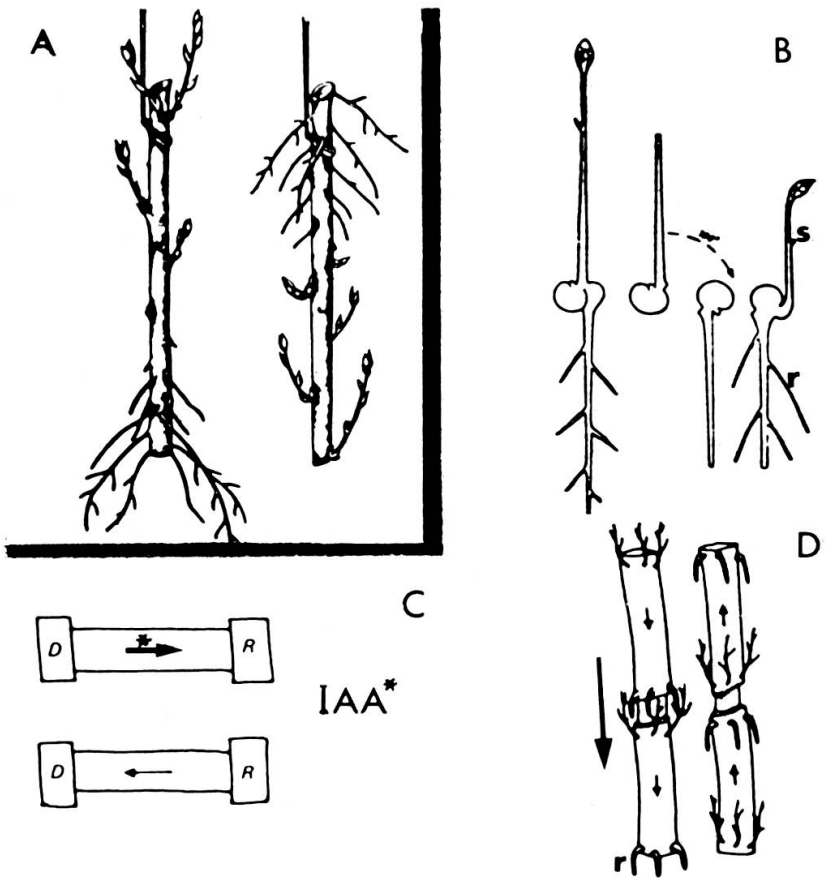


FIG. 29.

Polarity in green plants.

(A) In willow shoots: left, portion of a stem suspended in moist air in its normal root and shoot position; right, a stem similarly grown except in an inverted position. After Pfeffer, 1871, adapted from Sinnott, 1960.

(B) Reversal of polarity in etiolated pea seedling with epicotyl decapitated, inverted, and grown in water; roots (r) now emerge from the epicotyl and shoot(s) from a cotyledonary bud. After Castan, 1940, adapted from Sinnott, 1960.

(C) Polar transport of auxin from a donor agar bloc (D) to a receiver (R). Hormone (IAA*) movement occurs in the same direction as the tissue's polarity (starry arrow). From Sachs, 1984.

(D) Polar regeneration of girdled stem cuttings. The original direction to the root (r) marked by arrows; this polarity can be independent of gravity and the location of cuts and girdles. From Sachs, 1984.

it comes as no surprise to discover that inversion of a cutting in a gravitational field for example does not lead to inversion of the polarity of regeneration''. It can thus be concluded from the ivy experiment that polarity had been remembered and not reversed during the years of growth in the inverted position, despite the fact that presumably during that time the segment of the leg had functioned in a competent manner. This experiment, at first sight highly mysterious in its outcome, in fact confirms the notion of polar gradients of hormones as we shall see below (c^4).

c^4 *Polar auxin transport and tropic curvatures*

Polar transport (Fig. 29 C)

In higher plants, the transport of the auxin is basipetally polar as first proven, in 1932, by van der Weij. The model proposed for this hormonal transport requires that auxin moves from cell to cell by transport across the plasma membrane and the intervening of cell wall rather than primarily through plasmadesmata.

The plant growth substance IAA must be transported basipetally from its sites of synthesis in shoot apices and young leaves to the subapical target tissues in which it exerts its many developmental effects. The current hypothesis describing the mechanism of this polar auxin transport is the "chemiosmotic hypothesis" proposed in 1974 by Rubery and Sheldrake. It includes: H^+ gradient-driven cytoplasmic auxin accumulation by diffusion of lipophilic undissociated IAA molecules and by carrier-mediated cotransport of IAA anions and H^+ ions; transmembrane efflux of IAA anions on a carrier preferentially localized at the basal end of cells in the transport pathway (Rubery, 1980-81). The polar efflux step can be blocked by a group of synthetic compounds examplified by naphtylphthalamic acid (NPA) which bind to a plasma membrane protein receptor. Quite recently, a group of flavonoids (quercetin, apigenin, etc.) have been shown to act as endogenous ligands to the NPA receptor and thereby to perturb polar auxin transport (Jacobs and Rubery, 1988).

In the chemiosmotic hypothesis of this auxin transport, the energy stored in the electrochemical gradient could be applied to polar transport if a carrier protein for auxin anions were preferentially located at the basal ends of cells in transport pathway. The structural basis of cell polarity would thus be an enhanced basal permeability to auxin anions brought about by asymmetrical distribution of carriers. In her review of this model, Goldsmith (1977) termed it the chemiosmotic polar diffusion hypothesis, predicting both quantitative and qualitative features of polar auxin transport in the context of the mathematics and diffusion processes.

In summary, active and polar auxin transport is performed by the cooperation of two specific molecular transmembrane processes at the plasmalemma: an accumulation of IAA into the cytoplasm is driven by a pH gradient and by the electrical membrane potential and an efflux of IAA through polarly distributed exit carriers. In vesicle systems IAA accumulation was also shown to be dependent on pH gradient even though rough estimates of IAA accumulation into vesicles yielded ratios higher that the H^+ gradients (Hertel *et al.*, 1983). However with some modifications, the chemiosmotic theory remains valid, and auxin accumulation but not transport — with efflux limiting — can therefore be considered as electrogenic.

A proton motive force has also been shown to exist between the cytoplasm (pH around neutrality, negative potential) and the cell wall (acidic pH, positive electrical potential). This finding has led its authors (Rayle and Cleland, 1977) to explain auxin action as implicating a stimulation of proton excretion and a subsequent acid-induced

wall loosening. A clear correlation between acidification and growth is however still lacking (see c^1).

Tropic curvatures

Graviperception in roots requires specialized cells, the statocytes endowed with a structural polarity which is a prerequisite for their function. These specialized cells are localized in the root cap and their polar organization is based on a proximal nucleus and distal ER cisternae. Statoliths — mostly containing amyloplasts — serve for the perception of the gravity stimulus.

In higher plants, polar structures in the parietal cytoplasm serve as stimulus transducers. The polarity of the statocytes could not be affected by fast rotating (55 rpm) clinostat — microgravity — treatment and would thus be determined genetically. However, in roots centrifuged (1000 g) in apical direction, stratification of the statocytes was produced but their graviresponse was delayed. This coincidence of restitution of polarity with the delay of graviresponse suggested that a contact between amyloplasts and the distal ER complex is a necessary precondition for graviperception (Sievers and Hensel, 1982). In growing roots tilted at 45° from the vertical direction, the preceding symmetric patterns become asymmetric: the ER complex is differentially compressed by the sedimenting amyloplasts, provoking a potential depolarization (DV_d) in the physically lower and hyperpolarization (DV_h) in the upper statocytes. These changes generate a net asymmetrical current pattern across the root cap, the site of graviperception. Eventually, the cellular asymmetry is transduced to the asymmetry of growth. Parallely, the change from symmetrical to asymmetrical pattern of current flow of positive ions (probably H^+) preceded the graviresponse, suggesting a connection between current flow and the transduction of information from the root cap to the elongation zone. According to Behrens *et al.* (1982), the connection between perception of the gravity signal and its translocation into physiologically effective information appears to be insured by alterations of the membrane potential of statocytes as probable consequence of a local ion gradient generated by the ER and transmitted to the plasmalemma (analogy with photosensor in retina cells).

When exposed to light, primary roots of a few plants respond by a downward curvature or positive gravitropism; this is because light-induced growth inhibitors accumulate in the lower part of their horizontally-oriented roots. Abscisic acid has been strongly implicated as one of the growth inhibitors in the georeaction (Pilet, 1977). It has further been shown that this downward curvature of horizontally-positioned roots may be the consequence of a redistribution of endogenous hormones such as auxin and abscisic acid inducing a differential growth between the upper and lower parts of the roots (Pilet, 1985). Plasmalemmal binding sites could recognize the informational signal of the hormones and control their polar transport from cell

to cell (Kende and Gardner, 1976). Changes occurring in elongating cells from the two sides of gravireacting roots have been investigated using protoplasts prepared from the extension zone of maize roots (Senn and Pilet, 1980). The zeta potential directly depends on the surface charges (ionization of surface groups of proteins, polysaccharides, etc.) of the plasmalemma and should thus reflect its characteristic properties. Increase in the negative potential was found to be higher for plasmalemma from the "higher" located cells than that from the "lower" located cells of gravistimulated roots (Pilet, 1985).

Gravitropic or phototropic curvatures of shoots have also tentatively been explained by the lateral movement of auxin which leaving one side should speed up growth on the other side. However, rather than moving to produce differential growth, as predicted by the so-called Cholodny-Went model, auxin could be differentially released, by an homeostatic reaction, from its conjugated forms on the upper and lower sides of a horizontal shoot (Bandurski, 1980). As working hypothesis for the geotropically-induced IAA asymmetry, Bandurski *et al.* (1986) proposed that the deformation of the cell's bioelectric field provoked by gravity-positioned charged organelles results in "transient potential changes across the plasmodesmata such that these structures open, and or close, resulting in an altered movement of IAA from the vascular stele into the surrounding cortical cells. If plasmodesmatal gating also results in heightened ion gradients from one side of the tissue to the other, a device would also be at hand for amplifying the original weak signal generated by cutting the cell's bioelectric field".

Non hormonal models of differential growth have also been proposed such as that of a proton gradient. This gradient and an influx of K^+ ions leading to osmotic changes have been implicated as a possible cause of gravitropism in sunflower hypocotyls (Mulkey *et al.*, 1981). The zone of H^+ efflux and therefore of auxin-induced increase in extensibility of the longitudinal walls is stimulated on the upper surface, that of positive gravitropic curvature, of horizontally-placed maize roots (Mulkey and Evans, 1981). It is thus possible that the H^+ efflux asymmetry from the elongation zone, associated with the gravitropic reaction, is caused by auxin redistribution. However, the evidence for a correspondance of pH and growth rate changes during gravitropically-induced curvature is not yet convincing (Digby *et al.*, 1982).

Asymmetrical distribution of Ca^{2+} ions has also been implicated in the process as suggested by the reversible loss of gravitropic sensitivity in maize roots after tip application of calcium chelators (Lee *et al.*, 1983; Evans *et al.*, 1986). Further work should therefore be devoted to the calcium (calmodulin)-enhanced movement of auxin to the lower side of the elongating zone to understand the remaining steps of the gravitropic response.

A family of auxin-regulated RNAs from soya-bean has been found to begin to accumulate within 2.5 min after application of auxin. According to a recent report

by MacClure and Guilfoyle (1989), gravistimulation rapidly alters the distribution of these RNAs in responsive organs and the presence of the RNAs is highly correlated with cell extension, suggesting that auxin-regulated gene expression is involved in the response of plants to gravity.

c^5 *Flowering shoots*

The reproductive stages of the life cycles offer striking examples of basic developmental processes among which polarity, pattern formation, and determination. Thus polarity during macrospores and embryonic development is determined at an early stage (Bloch, 1943).

Floral differentiation is a process which includes all events/processes occurring in the meristem or the cells of the meristem which pertain to the production of the flower (inflorescence) as well as its maturation and senescence. Thus, "floral differentiation" is an all-inductive process (McDaniel, 1984*b*). Single algorithms or sets of rules can be used to describe such complex biological patterns which control the inflorescence development — distribution patterns of branches and flowering structures — by either timing or positioning (basi-meso-acrotonic) mechanisms (Lindenmayer, 1984). Flowering constitutes an end-point for the meristem which gives rise to the flower, and the frequent enlargement of the shoot apex during the changes to the reproductive state is paralleled by ultrastructural changes (Nougarède, 1967; Auderset and Greppin, 1977).

The dimorphism of branches presented by certain plants (*Phyllanthes* sp.) results from the plagiotropic orientation of a lateral bud meristem. It was shown to depend on the meristem of the orthotropic axis which formed it and led Nozeran (1984) to draw a suggestive parallel between this process and that of an organizing center ("organizer") in an animal embryo. The informational controls regulating the positioning of floral parts at the apex differ, at least quantitatively, from those which regulate the positioning of leaf primordia (Carr, 1984). Interestingly, "floral induction and fern leaf development appear to be analogous to the example of neural induction in amphibians where induction of competent tissue leads to the expression of a specific development fate" (McDaniel, 1984*b*).

In the conversion from vegetative growth to floral differentiation, the control of the axis of cell elongation appears to be critical. Reorientation of cell elongation axes or polarity shifts have been considered as related to the initiation of lateral plant organs (Green and Poethig, 1982). "In order to understand processes like floral determination one will therefore have to know how polarity shifts are controlled" (McDaniel, 1984*a*).

The conversion of the apical meristem from the vegetative to the reproductive condition is a dramatic example of the switching of a developmental pathway (Bernier

et al., 1981). In photoperiodic plants, this redirection of morphogenesis at the meristem is under environmental control. Exposure of these plants to an appropriate daylength causes formation of a floral stimulus. In the leaves and subsequent translocation of the stimulus to the apical meristem, and upon arrival of the stimulus, the meristem stops making leaves and begins to produce bracts and flower buds (Bernier, 1971). Photoperiodic induction of flowering mediated via phytochrome causes the leaves to transmit a still unknown signal(s) or floral stimulus to the shoot apical meristem(s) which responds by forming a flower.

Recently, floral morphogenesis could be produced in thin layer tissue cultures of *Nicotiana tabacum* (van den Ende *et al.*, 1984). It was shown that the hormone naphthalene acetic acid (NAA) and benzyladenine (BA) in different concentrations affect the formation and distribution of flower buds and callus formation. Polarity occurred in the formation of both callus and flower buds, the degree of which depends upon the hormone concentrations. The suppression of the polarity of flower bud formation by high concentration of NAA was explained by the flooding of a natural NAA gradient in the tissue.

Interestingly, gradients of flowering substances or physiological states were evidenced in cells of leaves which, when placed in an adequate culture environment, produce either vegetative buds or flowers, depending on the location of the explanted cells in relation to the inflorescence (Tran Thanh Van, 1973) suggesting a polar pattern of distribution of the floral inductive factor(s). Translocable flower-inducing substances and flower-inhibitory substances produced from photoinduced leaves presumably interact at the stem apices to determine whether the apex remains vegetative or undergoes evocation and flower formation (Lang, 1980). In the short-day *Sinapis alba* the flowering principle would consist of at least two components, one of which induces an increase in mitotic activity at the stem apex (Bernier *et al.*, 1977). Clearly something moves from photoinduced leaves to stem apices that triggers the onset of flowering (Cleland, 1982). This was already suggested by the circadian changes of membrane potential occurring in the light-stimulated bioelectric response of spinach leaves which can be used as indicators of floral induction (Greppin and Horwitz, 1975).

B. Animals

The idea that axial polarity may be an expression of an underlying cellular polarity is also consistent, to some extent at least, with the fact that the former is often associated with an electrical polarity and can be altered by imposed electric fields (Barth, 1934).

Spatial organization could also be accomplished by graded distribution of substances termed morphogens (Boveri, 1901; Child, 1929, 1941). Wolpert (1969,

1971) developed this idea further into the concept of positional information. He pointed out that the size of an embryonic system is small when determination occurs (order of 1 mm or 100 cells across). Diffusion combined with local production and destruction at opposite ends can form a gradient of this size within a few hours (Crick, 1970).

Morphogens are signal substances emitted from organizing centers controlling position and polarity of cell structures. Such signal molecules are presumed to exist in embryos and to be involved in establishing the spatial pattern of cells during development. The idea is that cells respond to different concentrations by adopting different pathways of differentiation and so produce the characteristic anatomy of the organ concerned (Slack, 1987*a*). Some gradient systems of morphogens are based on diffusion of chemical substances from a more metabolically active "source" at one tissue boundary to a lower point, or "sink ", at the opposite boundary. An active factor from the source diffuses in a polarized manner and is either lost or used up at the sink.

The diffusion of a morphogen between a source and a sink considered in the illustration of Wolpert's ideas, is only one of the numerous candidates potentially responsible for the establishment of positional information and for the subsequent pattern formation. As shown by Crick (1970), the time necessary for a morphogen to diffuse through a field of critical dimensions is compatible with experimental data. The diffusion coefficient appears to be of the order of 0.8×10^{-6} cm^2 s^{-1}, which is about the value of the diffusion coefficient in solution of low-molecular-weight substances such as cyclic AMP. A very instructive illustration of Wolpert's ideas is provided by the so-called "French flag problem" (Nicolis and Prigogine, 1977): consider a one-dimensional field, the position of cells can be defined with respect to one of the extremal points (unipolar system) or with respect to both (bipolar system). In the case of bipolar fields, the state of a cell is determined by two morphogens and is influenced by the state of *both* neighboring cells. Patterns displaying size variance can be generated (Garay, 1977). There exists also a possibility of bifurcations leading to a uniform distribution of the morphogens, as well as of wavelike behaviour (Babloyantz, 1977; Babloyantz and Hiernaux, 1974).

Concentration gradients of chemical morphogens are believed to act across small fields of embryonic cells where different concentrations of morphogen provide "positional information" that determines the future behaviour of the cells. Until recently however, while the logical case for such mechanisms was impeccable, the biochemical evidence was inferential at best. The position has now changed (Slack, 1987*b*) since evidence has been reported strongly implicating gradients of retinoic acid in the specification of antero-posterior digit pattern of the chick wing (Thaller and Eichele, 1987).

To account for pattern regulation (e.g. regeneration) the "polar coordinate model" (PCM) has been proposed (French *et al.*, 1976). It is a formal representation

of a two-dimensional coordinate array of positional values with a circumferential and a radial components. This PCM model has been successful in describing and predicting pattern regulation in imaginal discs, insect legs, and vertebrate limbs. However, remarks have been raised against phase singularities and proposals made for an alternative PCM model without polar coordinate (Lewis, 1981).

1. Monoaxial patterns (Antero-Posterior (A/P) polarities)

Polar patterns are not confined to organisms that have developed in the ordinary way by growth from a reproductive unit such as spore or egg but are found in what are essentially organismic communities.

a) *Mycetozoa (slime molds)*

In the model cellular slime mold *Dictyostelium discoideum,* considered here as a mycetozoan for organizational reasons (protomodel of hydrozoa), the vegetative individuals are tiny myxamoebae which are polarized in their response to changes in chemotactic gradients (V.B.5a). At the end of vegetative growth, some thousands of these become aggregated into a pseudoplasmodium where each retains its individuality. This colonial structure shows a polar organization, for the terminal portion of it can be grafted to the decapitated apex of another pseudoplasmodium, though not to the base. The tip is evidently the dominant region, for if grafted to the side of a pseudoplasmodium it will withdraw from it a group of individuals and start out as a new unit. The sorocarp that ultimately develops has a vertical polar axis and, in some species, lateral axes as well. Polarity in organisms like these appears to be a property not of the individual cells but of the aggregate that they form (Fig. 30 A, B; Raper, 1940; Loomis, 1982).

In the pseudoplasmodium of *Dictyostelium discoideum,* only two cell types are clearly recognizable: the prespore cells, which will eventually form the spores at the top of the fruiting body, and the prestalk cells, which will eventually form slender stalk bearing the mature spores at the apex. Cells at the tip appear to move downwards into this tube and differentiate into stalk cells, i.e. they vacuolate and become surrounded by a rigid cellulose cell wall. When the newly formed stalk makes contact with the substratum, the cells surrounding the stalk are lifted into the air. After the stalk has reached a certain height, these remaining cells become spores. A small group of cells, referred to as the tip, determines the direction of morphogenetic movement and the polarity of the pattern of the two cell types (Schaap, 1986). The tip functions as the classical organizer, a feature which is generally thought to act in embryonic development and tissue regeneration.

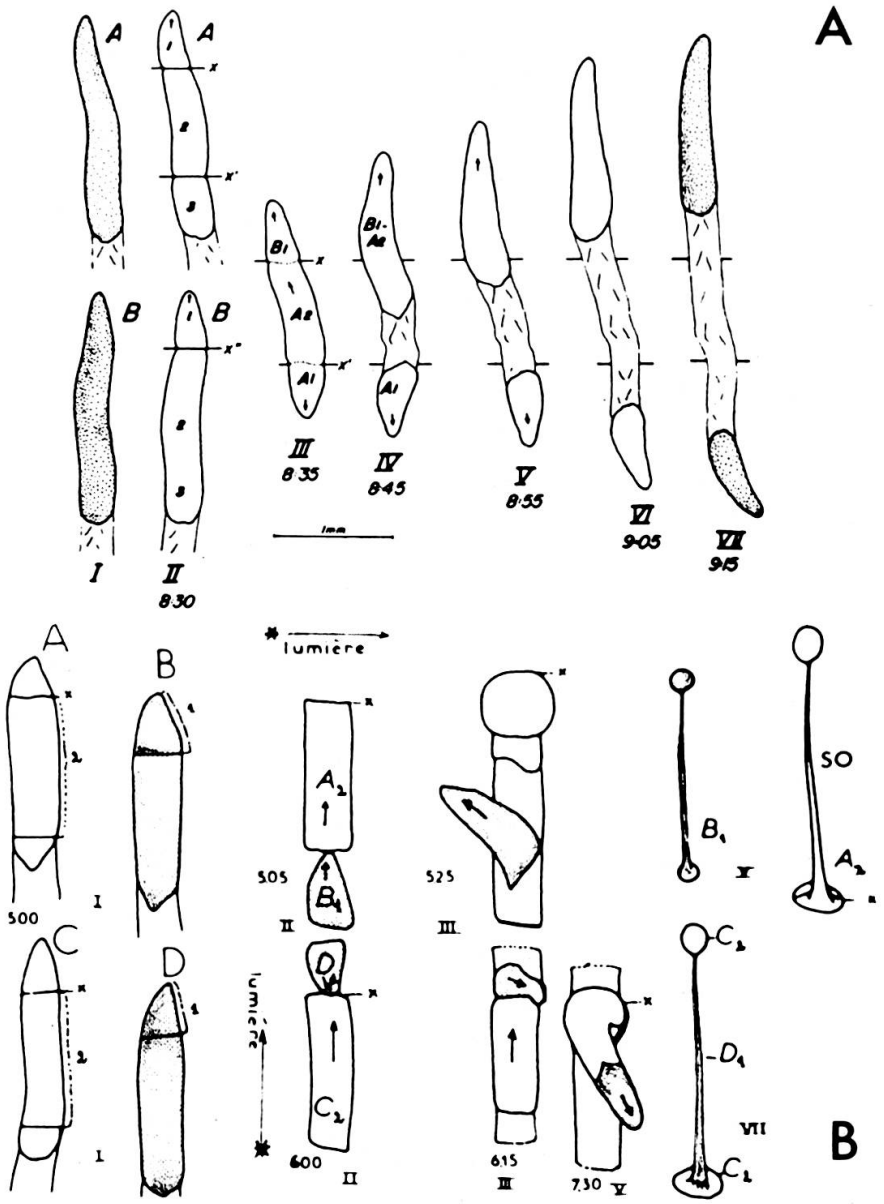


FIG. 30.

Polar axiation in the slime mold *Dictyostelium discoideum*.

(A) Regeneration experiment by Raper (1940) demonstrating polarity after grafting (A) an apical piece (B_1) of a pseudoplasmodium in close contact with the apical end of another, resulting in coalescence of the two pieces (III); (B) by contrast, there is *no* coalescence when the apical piece (A_1) is placed next the basal end (X') either of the same pseudoplasmodium or of another, the terminal piece (A_1) moves off independently (IV-VII). This experiment demonstrated the dominance of the slug tip. From Sinnott, 1960.

(B) Further demonstration of the polar behavior of apical and non apical fractions of pseudoplasmodia (A-D), certain of which (B and D) were stained by the red bacterium *Serratia marcescens* (darkened). When the two grafted pieces have the same polarity (arrows) two stalked fruiting bodies (sorocarps, so) are light (*)-produced while an inversely polarized piece (D) regenerated only one sorocarp. From K. B. Raper, 1940 in J.-P. Larpent "De la Cellule à l'Organisme», Masson & Cie, Paris, 1970.

In *Dictyostelium* the two types of cells can be distinguished biochemically on the basis of their stainability, specific protein content, antigenic properties, biosynthetic capacities, etc. Presumably the differentiation into prespore and prestalk cells reflects the activation of different sets of genes which then provide each cell with the specific templates needed to carry out its own differentiation. The underlying biochemical differentiation manifests itself morphologically, as a specific type of membrane-bound vacuole that is not found in prestalk cells (Karp and Berrill, 1981). The axially polarized slug formed from the aggregated myxamoebae (Fig. 30 A) moves according to a "squeeze-pull" model proposing that prestalk cells are engines and prespore cells are the cargo (Williams *et al.*, 1987).

A number of mechanisms have been proposed for the generation of two cell types in *Dictyostelium* including the choice of cell type being determined by a cell's position within the aggregate (Krefft *et al.*, 1984), or by other, non positional factors. The hypothesis that positional factors determine cell type choice is supported by the observation that a dissected anterior tip will form a new, smaller slug in which prespore cells are formed by conversion of prestalk cells (Raper, 1940). Interconversion can also be seen with purified populations of prestalk and prespore cells (Weijer and Durston, 1985).

Gomer and Firtel (1987) have examined the developmental fate of individual cells in a system that allows *Dictyostelium discoideum* cells to differientiate in the absence of aggregation. Their results show that the propensity of single amoebae to differentiate into either prespore or prestalk cells occurs by a cell-autonomous mechanism dependent on the cell's position in the cell cycle at the initiation of development.

Of primary interest to developmental biologists is the nature of the agents that cause a cell or group of cells to proceed along one path of differentiation as opposed to another (Karp and Berrill, 1981). In some cases, specific chemical substances produce complex responses in competent cells, i.e., cells capable of responding to the stimulus. This is clearly the case for the aggregation response by starved amoebas to cyclic AMP. More recently, another such "on-off switch" has been found to operate at a later stage in slime-mold development. It is evident that low-molecular-weight molecules such as cyclic AMP or ammonia are not substances in which developmental information can be stored. Rather, each serves as a specific trigger to elicit a preprogrammed reaction within the responding cells (Williams, 1988).

One of the consequences of the multicellular state in a pseudoplasmodium, or in any other cell mass, is the potential for intercellular, possibly polarized communication that results from cell contact. The outer edge of all cells forms a complex structure capable of transmitting and receiving a wide variety of stimulatory signals. Cells kept in isolation, and therefore free of cell contact, cannot undergo differentiation into prespore and prestalk cells.

The pattern of initial prespore differentiation suggests the existence of positional cues: in late aggregates, prespore-specific proteins and prespore vacuoles appear — approximately concomitant with tip formation — in the basal/central part of the aggregate and remain absent from the tip region. During subsequent slug formation, prespore-specific gene products remain confined to the posterior part of the slug. The results of such experiments would seem to demonstrate that the induction of prespore differentiation is not necessarily position-dependent (Schaap, 1986). However, it should be noted that, in species other than *D. discoideum,* position-induced (re)differentiation is more conspicuous.

The induction of stalk-cell differentiation is also position-dependent during normal development and, in *D. discoideum,* starts at the ultimate tip and at the base of the fruiting structure. The cellulose stalk tube in fruiting bodies is formed just before stalk-cell differentiation. A differentiation inducing factor (DIF) has been shown to induce such stalk cells (Kay and Jermyn, 1983). The DIF may be related to other morphogens (Watts, 1987) such as the low molecular-mass hydrophobic hexanone derivatives of the retinoic acid type.

b) *Protozoa*

The cellular polarity of a ciliate such as *Paramecium* is shown by a terminal cytostome. At cell division, kineties (rows of basal bodies or kinetosomes) elongate by multiplication of kinetosomes, the cell constricts in its middle and polar formation of a new oral apparatus produces a new daughter cell.

Analyses of cortical patterning of kinetosomes and kineties in ciliates protozoa show that there are two types of developmental processes responsible for the positioning of these organelles. One is the propagation of ciliary rows through localized addition of new ciliary units along the axis of an existing row (Karp and Berrill, 1981). The cortical pattern of ciliary rows seems to be completely independent of the nuclear information. When two differently striped cells of *Tetrahymena* exchange their nuclei, they do not change their cortical pattern. It is perpetuated by the cortex alone.

What controls the polarity of the ciliation wave? Is it the intrinsic polarity of the ciliary row, or is it some other polarity superimposed upon the ciliary row?

There are two systems of polarity in *Tetrahymena* which are both expressed with ciliary rows. This story begins historically with *Paramecium,* in which Beisson and Sonneborn (1965) were able to induce cells to rotate a few of their ciliary rows 180°, following which the paramecia actively maintained and propagated these rows in their inverted state. This demonstration was repeated in *Tetrahymena* over a decade over. Hence there exist two superimposed systems of polarity: "a local one internal to the ciliary rows governing (for example) the direction of nucleation of new basal bodies, and a second large-scale cellular polarity that is independent of the orientation of ciliary rows but nonetheless is able to impose its influence by controlling the time and place of formation of new ciliary structures" (Frankel, 1984).

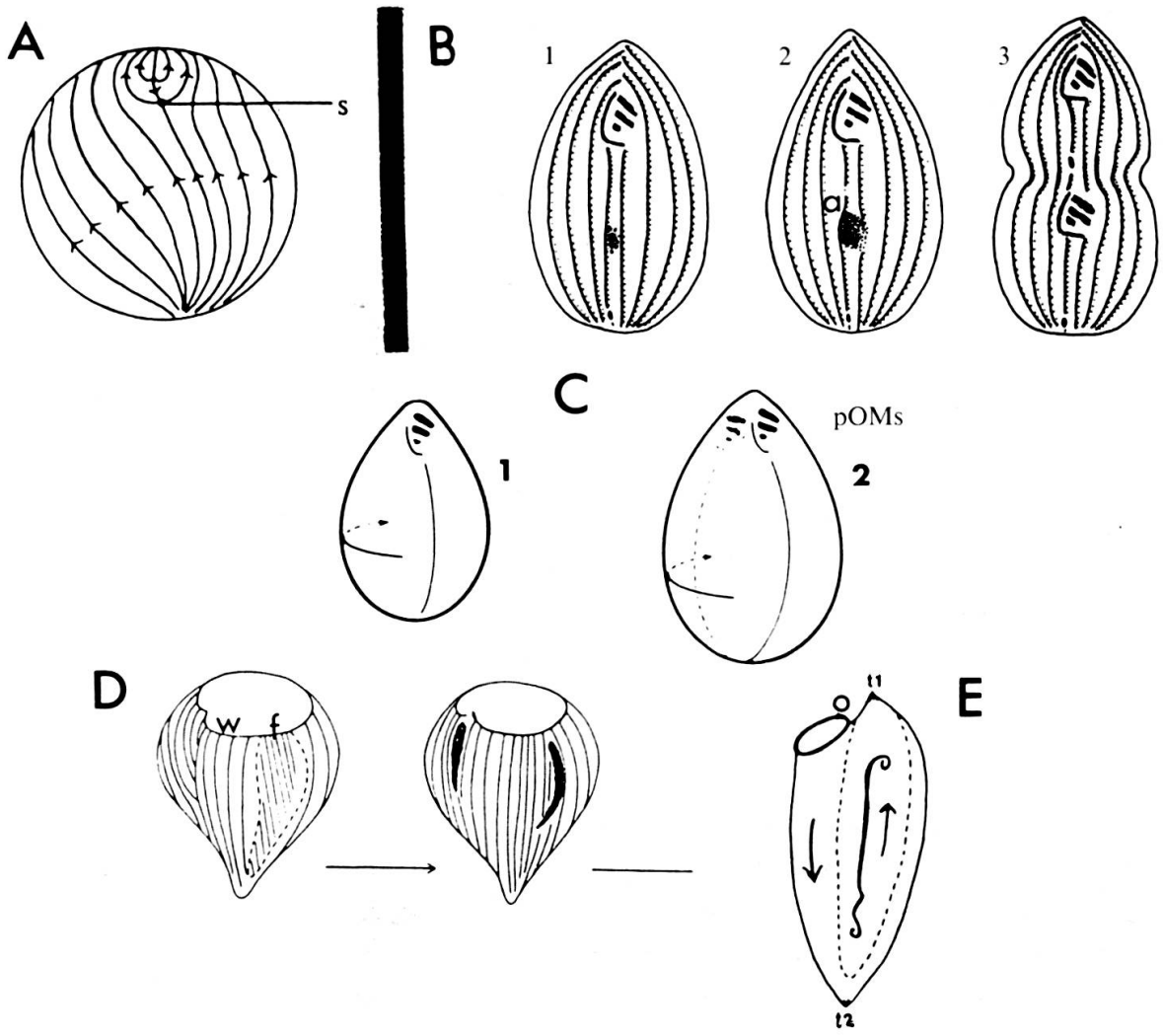


FIG. 31.

Polarity controls in *Protozoa.*

(A) Model of ciliary meridians: the arrows define the direction of morphogenetic field which establishes posterio-polarity saddle(s) point. (B) Cell division stages in *Tetrahymena:* (1) basal bodies initiating a new oral ciliature; (2) anarchic (a) field formed; (3) oral membranelles assembled. From Sleigh, 1973, adapted from Goodwin, 1976.

(C) Schematic lateral view of transparent, nondividing cells of *Tetrahymena* illustrating the geometrical arrangements of oral structures and oral meridians in non-*janus* singlets (1) and homopolar doublets (2) resulting from the fusion side by side of two cells. The resulting two semicells of the parabiotic, homopolar doublet are similarly antero-posteriorly aligned. The demonstrated clonal persistence was the original basis for Sonneborn's contention (1963) that preformed cell structures play an essential role in cell heredity (Fauré-Fremiet, 1948, and Sonneborn, 1963, in Frankel *et al.,* 1984). pOMs = primary oral meridians. Adapted from Frankel *et al.,* 1984.

(D) Double oral regeneration in *Stentor* resulting from grafting an extra fine (f)-stripe zone into the wide (w)-stripe region of a decapitated cell; the new oral primordium is polarly reversed. From Tartar, 1962, adapted from Karp and Berrill, 1981.

(E) Experimental demonstration of the importance of the posterior region of the *Stentor* cell induction of an oral spiral. This spiral is formed at both ends (t1, t2) of the oral primordium (o) following excision of a nonnucleated segment from the region of the contrast-zone and reimplantation following 180° rotation about the antero-posterior axis. The arrows show the intrinsic polarity of the host and of the implant. Adapted from Frankel *et al.,* 1984.

At cell division in *Tetrahymena,* observation of polar disturbance by the anarchic field of proliferating basal bodies led to the conclusion that "the oral apparatus arises at a saddle point in the control field, where polarity vanishes" (Fig. 31 A, B). The anarchic field disturbs the polar organisation of the local ciliary meridians, and then "this oral field becomes organised into the typical ciliature of the mouth" (Goodwin, 1980).

The restriction of the basic pattern to the cell surface, together with the fact that the organism has polarity so that the anterior (or north) pole is different from the posterior (or south) pole, is sufficient to select from among those functions satisfying Laplace's equation (more generally, Poisson's equation) on the sphere a unique one which describes the basic polar organization of the cortical field (Goodwin, 1980).

Cortical structures also propagate in a stable hereditary manner in *Stentor* as demonstrated by the classical, double *Stentor* experiment (Tartar, 1962): a cortical segment from a fine-stripe cell grafted into the wide-stripe region of another decapitated *Stentor* cell results in double oral regeneration, one from the host and one from the graft primordium (Fig. 31 D, E). Each cell division produces two deranged daughter cells. Moreover, decapitation of the double *Stentor* is followed by double regeneration. The genes have therefore not changed, only the cortical structures. Yet cortical structures are also genetically controlled (Loomis, 1986): homozygous cells of the two-faced mutant of *Tetrahymena janus* (*jan/jan*) show two oral apparatuses on opposite sides of the cell in mirror-image orientation (Fig. 31 C). The *jan* allele would modify a reference dorsal border, the dorsal side of the mutant cells being transformed into a reversed ventral one. "Analysis of symmetry-reversals that result from altered gene action and those that result from geometrical anomalies in wild-type cells promise to shed light on the mechanism of cellular pattern determination" (Loomis, 1986).

c) *Hydrozoa*

The fresh-water polyp *Hydra,* a coelenterate of about 5 mm length, of about 100,000 cells and of about 12 types (Saier and Jacobson, 1984), has been a classic model for polarity studies since its discovery more than two centuries ago, when Abraham Trembley of Geneva first succeeded in cutting the small animal in two and observed the regeneration of the amputed parts. With a hair, he could pull the creature inside out, and subsequently observed its recovery to the normal state. The memory of this pioneer of polar regeneration studies (Fig. 32 A) has been honored for the two hundredth anniversary of his death by a special volume of the Archives of Sciences (Lenhoff and Tardent, 1985).

The major changes of form in hydra include the developments of: tentacles during regeneration and budding; new and complete axis during budding; partial secondary axes resulting of induction by grafts (Tardent, 1963; Webster, 1971). Along

its main axis, a hydra may be regarded as a bipolar field, if we define with Wolpert *et al.* (1974) a field as "that set of cells which have their position specified with respect to the same coordinate system or boundary regions". The hydra field thus is bipolar, since the two boundary reference regions appear to be the head and foot ends. As for the variation in positional value along the hydra, it may be represented by a gradient in some cellular property which decreases steadily from the head to the foot end (Wolpert *et al.*, 1974).

The metabolic origin of polarity in hydroids (*Hydra, Tubularia*) has been studied by Child (1941) and has provided him with the conceptual basis for the development of his theory of metabolic gradients, particularly of oxidative metabolism, as the underlying cause of axial order. By the use of inhibitors and dyes, this author could suggest that metabolic activity is the highest at the hypostome while decreasing regularly towards the basal disk of the hydra. Child's ideas have been reviewed by Webster (1971) and as commented by Goodwin (1976) "they remain as a foundation for much thinking about the dynamic nature of polar order in developing systems".

More recent experiments of polarity reversal, with nerve-free hydra, have suggested that hydra polarity is determined by epithelial cells (Marcum *et al.*, 1977). Such suggestion rejoins previous proposals that it might be due to either a metabolic and transport polarity or to a structural asymmetry. Such structural basis for polarity has been ascribed to polarized motions of axially aligned muscle processes (ref. in Marcum *et al.*, 1977, see also Fig. 32 B).

Complete organismal regeneration is possible from a very small fragment even though limited to a minimal size. If a section is cut from the central body part, a new head (hypostome and tentacles) will regenerate distally and a new base proximally, preserving the original polarity of the tissue. Polarity reversal can be produced in an electrical field or by grafting a hypostome proximally and removing it several days later (Goodwin, 1971). During regeneration, multipolar budding forms are often produced and mutants have been found which do not reproduce by budding (Lenhoff *et al.*, 1969). Other mutants, such as regeneration defective strains (Achermann and Sugiyama, 1985) as well as chimeras have been profitably used to explore the gradient underlying pattern formation in *Hydra* (Achermann, 1985).

Two developmental gradients of morphogens play a role in establishing the position of a regenerating head: the head *activation* gradient due to a relatively stable property of the tissue distributed monotonically, with tissue at the upper end of the body column having a greater ability to form a head and the tissue, further down the body column (MacWilliams, 1983) and the head *inhibition* gradient which is also monotonically distributed but very different in character (Fig. 32 C). These differentiation patterns were presumably thought to be controlled by gradients of diffusible molecules or "prepatterns". This concept of diffusible patterning substances was originally proposed by Wolpert (1969), and was later incorporated into reaction-diffusion models by Gierer and Meinhardt (1972) and MacWilliams (1983).

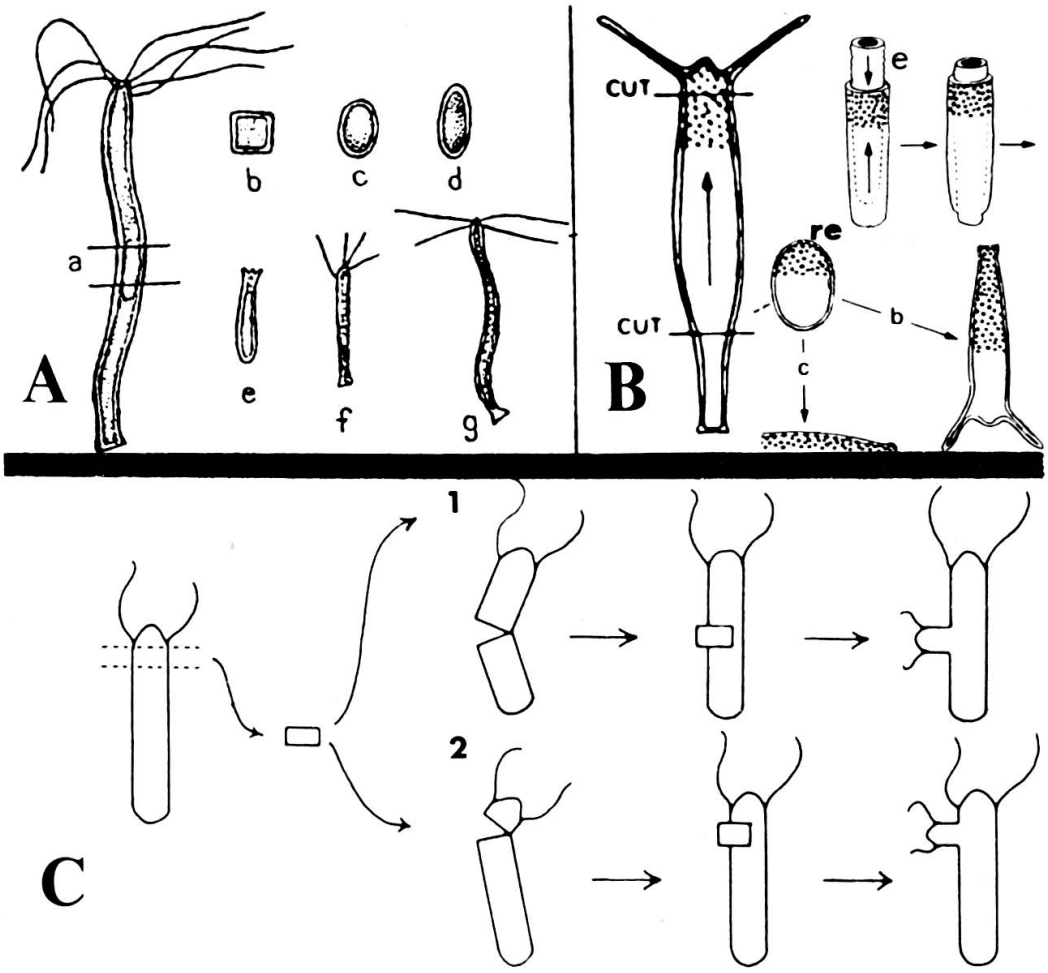


FIG. 32.

Polarity controls in Hydra.

(A) Classical Hydra regeneration experiment by Morgan, 1904: a = fragment cuts; b-g = regeneration steps according to normal apico-basal polarity.

(B) Influences of ecto- and endoderm in determining the axial polarity of *Hydra attenuata* Pall: ectoderm isolated from endoderm (e) and reassembled with opposite polarities (arrows); carbon marks indicate the former distal region of the ectoderm; in regeneration products among the reassembled (re) specimens a few (b) obeyed to the polarity of the endoderm. In (a, not shown) regeneration occurred according to the axial polarity of the ectoderm; in (c) the new axis is perpendicular to the original body axis. Adapted from I. Schmidt and P. Tardent, Wilhelm Roux's Arch. 191: 64-67, 1982.

(C) Transplantation experiments with *Hydra attenuata:* (1) transplant (donor) from head zone tested in the middle of a Hydra receptor (acceptor). The more transplant tested there originates from zones toward the tail zone, the less lateral head formation occurred, thereby demonstrating the existence of an axial head *activator* gradient. (2) Transplant (donor) from head grafted in head region of the acceptor resulting in a minimal (10%) head lateral growth; the closest to the tail is grafted the transplant, the more (ad 85%) is lateral head formation, thereby demonstrating the existence of an axial head *inhibitor* gradient. Adapted from figures by Bode *et al.*, 1987.

The basis of the activation gradient could derive from an intrinsic cell polarity by such mechanisms as directed morphogen transport or electrical conduction. However, patterning in hydra was experimentally showed to be more complex than

a single series of appropriately aligned north and south magnetic poles (Bode and Bode, 1984). In one experiment, rings of the body column were flipped over and then grafted back together again. Thus, each piece retained its original axial position, but had an inverted polarity. If polarity were simply a vector property, head regeneration should then occur at the basal end. Instead, it occurred at the original apical end, as expected if polarity were due to a *scalar* property.

A small peptide has first been isolated as morphogen responsible for the head-forming gradient (Schaller, 1973). It has been further identified as an 11-amino-acid peptide with pyroglutamyl and phenylalanyl residues (Schaller and Bodenmüller, 1981). This "head activator" can be generated by the reaction-diffusion mechanism and the profile of this neuropeptide has been found to "closely parallel the observed pattern of nerve cell commitment and hence the pattern of "free" head activator which was inferred from it" (David and Holstein, 1985).

The phenomenon of head inhibition is commonly assumed to be due to the steady-state production of diffusible "head inhibitor" by the head and its degradation in the tissues through which it diffuses. Recent results provide direct evidence consistent with the involvement of a diffusible hydrophilic and non peptide substance moving through gap junctions in head inhibition in hydra: "antibodies to the major rat liver gap junction protein recognize a gap junction antigen in hydra and are effective in eliminating junctional communication between hydra cells" (Fraser *et al.*, 1987). According to these authors "head inhibition might involve the cooperative influences of both diffusible and nondiffusible components, or an electrically driven passage of ions or small molecules through the gap junctions". These cellular structures are thus deeply involved in the polar patterning process in *Hydra* as extensively discussed by Bode *et al.* (1987).

In marine hydrozoa, the anterior-posterior axis of the planula larva has an integrative function (Teissier, 1931) and is frequently regarded as some sort of gradient running from one pole to the other in the embryo. Freeman (1981) has further shown that, after dissociation of blastula stage embryos into single cells, polarity can be entrained in the reaggregate by grafting a small piece of tissue from any part of an intact blastula to the reaggregate. The grafted cells then organize formation of an axis of symmetry in the embryo. This axis formation has tentatively been explained by chance events occurring during cleavage, translated by a principle of "self-increasing" elongation into an oxidative polar difference (Ostroumova and Belousov, 1971). More recently, this process of emergence of polarity in embryo cell aggregate has been analogized to the acquisition of magnetism by a metal (Freeman, 1981). When polarity is set up, this event can be regarded as "a process whereby a population of polarity dipoles in or near the cell surface become oriented in the same direction". Freeman's experiments have shown polarity to be entrained in reaggregated cells by grafting a small piece of tissue from an intact blastula to a reaggregate, a finding consistent with this dipole model.

As we have briefly described it above (p. 247), the body of work on hydra argues that diffusible morphogens produced primarily at either extremity of the animal play a major role in maintaining polarity. A morphogenetic head activator has also been found in the planula larva of *Hydractinia* (Müller *et al.*, 1977). However, this model of polarity founded on the reaction-diffusion models rather than on the oriented dipoles — disproved in *Hydra* by the scalar model — is probably not applicable to the marine hydrozoan embryo (Freeman, 1981).

2. Biaxial patterns (A/P + Dorso—Ventral (D/V) polarities)

a) *Worms*

The planarians or flatworms provide insight into how the antero-posterior and dorso-ventral axes are kept orthogonal to each other (which is not automatically the case if the patterns are formed by reaction-diffusion mechanisms). A condition for head or foot formation and thus for the re-establishment of an antero-posterior axis is a juxtaposition of dorsal and ventral tissues (Chandebois, 1979). A head or foot can therefore only be formed along the dorso-ventral borderline and never, for instance, within a purely dorsal region. This assures that both axes are orthogonal.

A head, a foot or both regenerate even in very small tissue fragments, indicating that systems which bear an organizing centre at each end are stable over an enormous range of sizes. The head field and the foot field cannot be independent from each other, otherwise they would not appear at opposite sides. The mechanism of mutual activation of two feedback loops suggests an appropriate coupling of a head-forming and a foot-forming system which assures that both structures are present in the system and that they appear at maximum distance from each other (Chandebois, 1979).

As in coelenterates, polarity is an important feature of flatworm regeneration. If a planarian is cut in half perpendicular to the antero-posterior axis, the anterior fragment regenerates a tail at the cut surface and the posterior piece regenerates a head. Regeneration occurs at both wound surfaces, not only by migration of coherent sheets as in coelenterates but also by the proliferation of undifferentiated cells at the cut surface into a blastema that differentiates new head or tail structures (Fig. 33).

Polarity seems to be intrinsic to the piece; changing its position by implanting it at different sites along the longitudinal axis does not alter its rate of regeneration. Anterior fragments regenerate heads more rapidly than posterior pieces, although this property varies from species exhibiting no head regeneration to others exhibiting no gradient where all fragments regenerate heads at the same rate (Grant, 1978).

Polarity expresses itself in many ways: when the wound surface of a posterior fragment is longitudinally cut into two components which are not allowed to fuse, each portion regenerates a complete head. A multiheaded monster may be produced

by making several parallel cuts at the surface. On the other hand, two heads may be fused to form one. The wound surface behaves as a morphogenetic field; that is, when disturbed, it regulates and restores a normal developmental pattern (Grant, 1978).

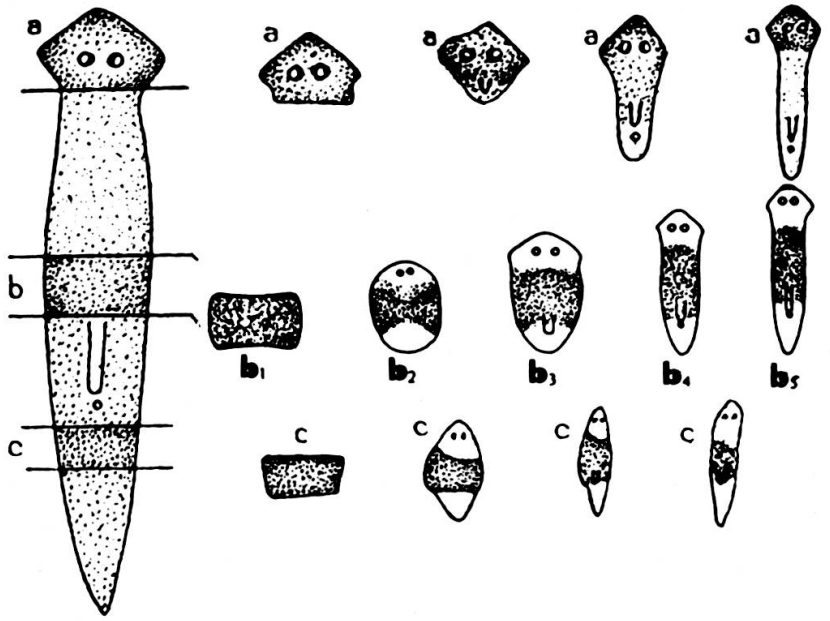


FIG. 33.

Polar regeneration in *Planaria maculata*.

(a) Monopolar regeneration of an excised head. (b_1-b_5) Bipolar regeneration of a transverse section from the middle. (c) Bipolar regeneration of a piece removed from a posterior segment of the body. Regeneration portions unstippled. From T. H. Morgan, Regeneration, 2d ed., New York, Macmillan, 1907.

Bipolar regeneration has been induced in the polychaete worm *Sabella* by means of exposure to colchicine. Colchicine inhibits all regeneration, but when colchicine-treated pieces are returned to normal seawater, a head may regenerate from each end. When such a two-headed piece is bisected, however, the anterior portion regenerates a tail posteriorly, and the posterior portion regenerates a head anteriorly. The original polarity is regained. The interpretation offered is that colchicine reversibly disrupts the normal array of microtubules in the nerve cord, i.e. it causes a temporary depolarization, while the production of neurosecretory granules is not inhibited.

How the epigenetic process responsible for temporal and positional control of cell determination in embryos operates remains a central problem of contemporary developmental biology. The transparent embryos of the round worm or nematode *Caenorhabditis elegans* provide a remarkable experimental system with which to approach such questions (Brenner, 1974; Kenyon, 1988). These embryos develop

rapidly, giving rise to first-stage larvae with only 558 nuclei. Moreover, this worm is convenient for genetic analyses, so that mutations perturbing embryogenesis can easily be isolated and studied for clues to normal developmental mechanisms (Hirsh, 1979).

The fate of each worm cell, including those of the nervous system from the fertilized egg to the adult, has been painstakingly traced by Sulston *et al.* (1983): the zygote undergoes a series of asymmetric divisions, each giving rise to a somatic precursor and a germ line cell. Most of these divisions take place in the direction of the antero-posterior polar axis, but several have dorsal-ventral or left-right components (Laufer *et al.*, 1980).

Boveri (1910) first showed that posterior zygote cytoplasm may act to prevent subsequent chromosome diminution in *Ascaris* embryos. This is consistent with the suggestion that germ plasm material may be important in either the determination of germ-line cells during early embryogenesis or the functions of germline cells during gametogenesis or both. The germ line of the nematode *Ascaris* is established by the inclusion of cytoplasmic components in certain embryonic blastomeres. Since then, nematode embryos have been regarded as classical examples of the "mosaic" type; that is, the fates of blastomeres are fixed at cell division by an unequal partitioning of cell constituents rather than by cell-cell interactions or environmental cues (Gurdon *et al.*, 1985; Emmons, 1987).

Graded signals are known to play a role in structural morphogenesis, in relation with positional information, and the spatial pattern of the inductive signal affects the morphogenetic outcome. In the case of germ cells, the inductive signal emanates from two somatic cells of the gonad. These cells, termed distal tip cells, lie at the distal end of the tubular gonad. If the distal tip cells are displaced from their normal location in the developing gonad, the polarity of the gonad shifts correspondingly so that germ cells nearest the distal tip cell are always mitotic, and germ cells further removed are in progressively later stages of meiosis or gamete formation. Therefore the distal tip cell is responsible for maintaining the normal spatial pattern of cell types in the field of germ cells. This must occur by a diffusible signal, since cells beyond those in contact with the distal tip cells are influenced.

b) *Molluscs*

There are polar lobes as in eggs of annelids. Polar lobes arise by a transient constriction that separates the anuclear vegetal region of the egg cytoplasm more or less completely from the remainder of the egg. The process starts during the maturation divisions, but the constrictions remain rather shallow. During first cleavage the constriction is very deep, resulting in a lobe that is connected by a thin stalk to the egg. This stage is called trefoil stage (Dohmen (1983).

The polar lobe content appears to be of crucial importance for development, as can be demonstrated by removing the lobe at the trefoil stage without injuring

the blastomeres. The egg continues cleaving but subsequent development is severely defective. As for the biochemical nature of the polar lobe determinants, it was found a higher concentration of guanosine triphosphate (GTP) in the polar lobe of *Nassarius* (van Dongen *et al.*, 1976).

The vegetal body of the polar lobe contains the morphogenetic determinants and the localization of the morphogens at the vegetal pole begins during oogenesis. The mechanisms that bring about this localization and provide the strong attachment of the determinants to the egg cortex are unknown but microfilaments might be involved. According to Dohmen (1983), "it remains to be established what significance should be attributed to these regional surface differentiations. They are formed at the vegetal pole of the egg, so they might be instrumental in establishing or maintaining the animal-vegative polarity of the egg without any direct relationship with the morphogenetic determinants present in the polar lobes". However, experimental data have shown that whole egg surface is capable of forming lobelike constriction, and the normal position of the polar lobes at the vegetal pole apparently depends on the correct positioning of the triggering signal. The mitotic apparatus may be instrumental in this process, but its presence is not absolutely required for the appearance of the lobe at the correct time and the correct place (Dohmen, 1983).

In normal development the polar lobes appear in synchrony with the cleavages. The stimulus for furrow formation in cell division is known to be provided by the mitotic apparatus (Rappaport and Rappaport, 1974) and observations suggest that the microtubules of the mitotic apparatus are involved in the initiation of lobe formation. However, the potential to form a polar lobe is apparently restricted to a limited part of the egg. If the vegetal part of an uncleaved egg of *Dentalium* is removed, the egg will not form a polar lobe at first cleavage. Polar lobelike protrusions can be induced anywhere on the egg by exposing fertilized eggs of *Ilyanassa* to isotonic $CaCl_2$ after second maturation division (Conrad and Williams, 1974). Similar effects are observed after iontophoretic injection of calcium, cyclic adenosine monophosphate (AMP) or ionophores (Conrad and Davis, 1980).

c) *Echinoderms*

The embryonic architecture of the sea urchin embryo begins with the initial polarity of the egg endowed with an animal-vegetal polarity (Fig. 34 B) originating before fertilization, far back in oogenesis (Guidice, 1986). In these embryos data relevant to determination along the animal-vegetal axis have been interpreted in a manner consistent with the idea that morphogenesis is regulated by a balance between reciprocal "animalizing" and "vegetalizing" gradients (Angerer and Angerer, 1983). Driesch's success in separating the early blastomeres of sea urchin embryos was a great boost for the argument of "isotropy". It seemed to refute the possibility that *any* predetermination of morphologic outcome can exist, at least up to the four-cell

stage. Driesch's result was a further blow to Weismann's proposal of nuclear differentiation and divergence. It remained for Wilson and Morgan, and for two entire generations to show that there are *not* two perfectly separable and distinct kinds of development, "mosaic" and "regulative" (Jeffery and Raff, 1983). There appears to be architecturally-differentiated domains of the egg cytoplasm, as evidenced by differential organization of the molecular cytoskeleton. They match the domains of those morphogenetic "plasms" so painstakingly traced out by the first investigators of cell lineage.

The location of the early blastomeres, and of the embryonic structures to which they give rise are fixed with respect to the animal-vegetal axis of the unfertilized egg. However, some cell lineages of the sea urchin embryo do indeed develop

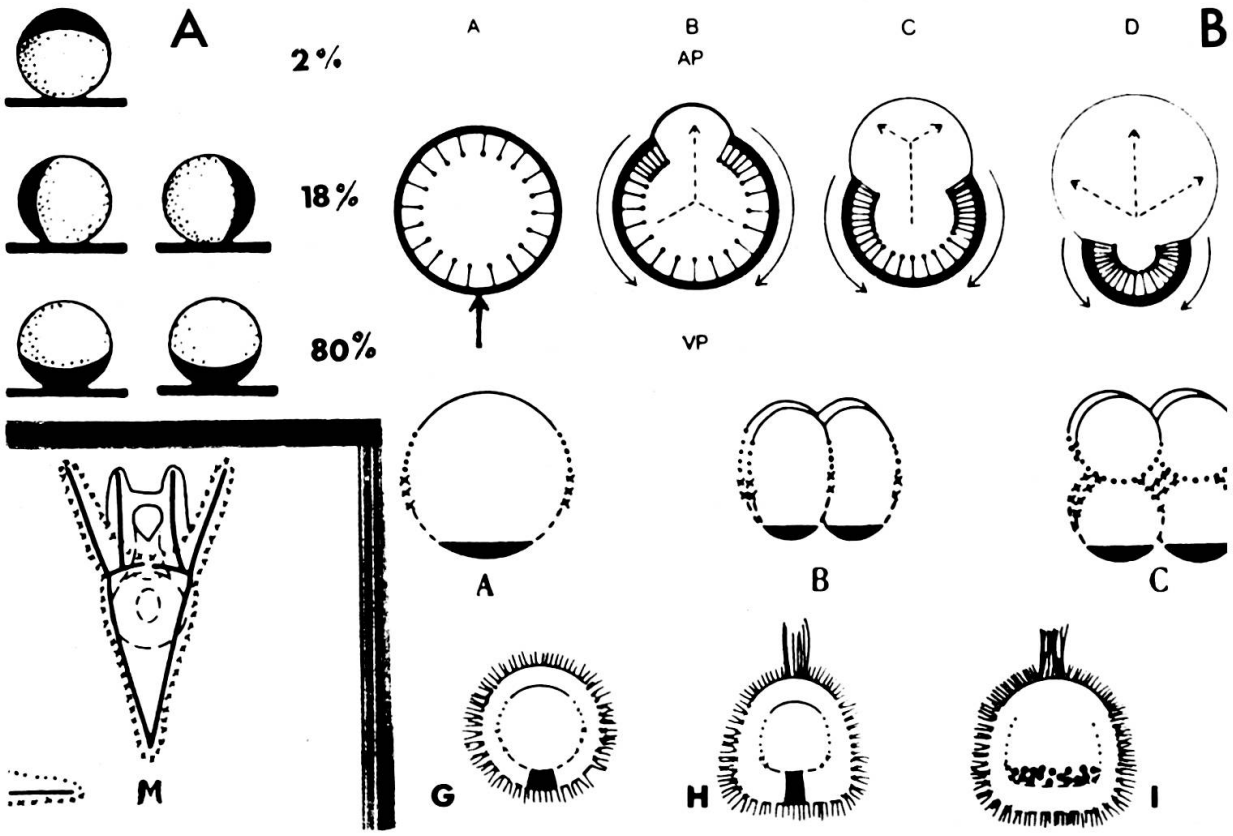


FIG. 34.

Polarization of Ascidian and Echinoderm eggs.

(A) Eggs of the ascidian *Boltenia* showing the direction of their orange myoplasmic crescent predominantly aligned (80%) against a Ca^{2+}-ionophore (A23187) — coated glass rod. Adapted from Jeffery, in Jeffery and Raff, 1983.

(B) Eggs of the sea urchin according to Jeffery's model (1983) in which the local calcium elevation at fertilization causes the plasmalemma to contract toward the point of sperm entry (arrow) in the vegetal hemisphere of the egg; further developmental fate of the various regions (A-I) of the sea urchin egg leads to the pluteus type of embryo (M) as based on Hörstadius, 1939, according to Grant (1978). AP = animal pole; VP = vegetal pole; crescent (black) = micromeres-forming region.

autonomously, but others are evidently specified for their pathway of differentiation by means of intercellular interactions or polarizations (Davidson *et al.*, 1982). In the eight-cell embryo there are four cells containing animal-pole substances and four containing vegetal-pole substances. This third cleavage is a determinate one; if the animal and vegetal halves of the embryo are separated, the animal half will give rise to an abnormal blastula-like larva with overdeveloped cilia, and the vegetal half will give rise to a different type of abnormal larva with an overdeveloped digestive cavity. It is clear, then, that the cytoplasm substances to which a nucleus is exposed must play a prominent role early during embryological development in activating some genes and repressing others. Because the egg cell itself contains different substances in different regions of its cytoplasm — whether these substances are arranged in a pole-to-pole gradient (as they apparently are in sea urchins) or in a less symmetrical pattern (as they are in frog and sea-snail eggs, with their gray crescents and polar lobes) — cells with different cytoplasmic environments for their nuclei are produced early in embryological development.

How the animal and vegetal halves have become so different in their abilities to differentiate in isolation? According to the theory of *double* gradient of Runnström (1975) and Hörstadius (1973), the direction in which a given cell of a sea urchin embryo will differentiate is under the control of two influences acting coordinately throughout the embryo. One of these influences (activities or substances of unknown nature) is at its greatest concentration at the animal pole; the other is at the greatest level at the vegetal pole. Each influence spreads out from its respective pole, decreasing until it has essentially disappeared in the region of the opposite pole. Since each diminishes in strength, each is considered to occur as a gradient, and together they form a double gradient. In this theory, the nature of differentiation of each part of the embryo is thus based on the relative levels of each member of the double gradient.

A correlation exists between the double gradient of oxidative metabolism and the double gradient of differentiation. The causal link between these two phenomena comes from the analysis of the metabolic gradient under conditions that are known to affect the morphogenetic gradients. The two appear to be linked. For example, lithium (LiCl) treatment abolishes the center of metabolic activity at the animal pole without affecting that of the vegetal region. Animalizing agents (thiocyanate SCN^-, trypsin) have the opposite effect and leave the embryo in possession of an intact animal gradient but lacking a counterbalance from the opposite pole. In each case, the ratios of the two influences are greatly disturbed, and the expected abnormal differentiation results (Lallier, 1975).

Developmental changes in the cell surface of the embryo have been examined and some antigens present in the egg found to become localized to the basal lamina or the matrix of the blastocoele (McClay *et al.*, 1983). Although the pattern of deposition is somewhat variable, and each antigen becomes localized to the wall of the

blastocoele, it should be noted that the same cells release the basal lamina material to the basal surface and other antigens to the apical surface of the embryo. This polarized expression of surface- or matrix-related antigens is a dominant theme for most of the antigens observed, and it will be of importance to learn how intracellular trafficking moves proteins in such a polarized fashion.

d) *Insects*

d[1] *Egg-embryo patterns*

The antero-posterior and the dorso-ventral egg axes are readily defined by the shape of the egg, yet the only specialized cytoplasmic organelles known to be localized are the polar granules of the posterior pole. They are included in a distinct clear zone of "pole plasm" and incorporated in the primordial germ cells, the first cells formed in the embryo. Nüsslein-Volhard *et al.* (1987) have recently shown that "the segmented pattern of the *Drosophila* embryo is organized by *two* activities localized at the anterior and posterior egg poles." The two morphogens, positioned at the anterior and posterior egg poles produce signals that spread toward the other pole. Thus this molecular prepattern of two opposing gradients is quite unlike the final fate map (Nüsslein-Volhard *et al.*, 1987).

A/P polarity is controlled anteriorly by the gene product of *bicoid (bcd)* and posteriorly by the gap gene *hunchback (hb)* repressed by the *nanos (nos)* gene. While a morphogenetic gradient underlies the specification of the anterior segment, positioning of the posterior one is determined only by local interaction between the gap genes (Hülskamp *et al.*, 1989; Irish *et al.*, 1989. At least twenty genes have been identified as playing crucial roles in D/V polarity of the early embryo (Anderson, 1987). It has been proposed that interactions among the products of the dorsal group genes are responsible for the specification of a morphogen gradient that ascribes unique positional identities to the different cells along the D/V axis (Anderson *et al.*, 1985). According to this model, the ventral-most regions of the early embryo contain the highest concentration of morphogen, with more dorsal regions containing progressively lower concentrations. Among the eleven maternally active dorsal group genes, *Toll* has been implicated as playing a particularly important role in the differentiation of the D/V pattern (Anderson *et al.*, 1985). However, the cloning and sequencing of *Toll* clearly indicate that it does not control D/V polarity by directly modulating gene expression (Hashimoto *et al.*, 1988). The *dorsal* gene has been recently cloned, and nucleotide sequence analysis reveals extensive homology with the avian oncogene v-*rel* (Steward, 1987; Levine, 1988).

Three kinds of mutations can disrupt the developmental process in *Drosophila*: maternal effect mutations, segmentation mutations and homeotic mutations.

Maternal effect mutations

Certain of these mutations influence the spatial polarity of the embryo and mutants are known which affect either antero-posterior (A/P) polarity or dorso-ventral (D/V) polarity.

A major determinant of A/P pattern is the product of the *bicoid (bcd)* gene (Frohnhöfer and Nüsslein-Volhard, 1986; Nüsslein-Volhard *et al.*, 1987; Driever and Nüsslein-Volhard, 1988). Like many other maternally expressed genes, *bcd* is transcribed in the ovary in specialized cells which form a cluster around the future anterior pole of the developing oocyte and the *bcd* RNA becomes localized at the anterior pole of the oocyte by virtue of its site of entry. Recent results of MacDonald and Struhl (1988) support the proposal that *bcd* transcripts are selectively recognized and trapped as they enter the anterior tip of the oocyte. A discrete *cis*-acting signal is responsible for this anterior localization of *bicoid* mRNA. At the same time that a stable graded distribution of *bcd* protein has formed from the anterior pole, it is assumed that an *osk*-dependent activity, probably, the product of the *nannos (nos)* gene has become inhomogeneously distributed in the posterior part of the embryo (Ingham, 1988; Fig. 35 A, B).

Among the maternal effect genes, at least twenty are known to control dorsal-ventral polarity (Nüsslein-Volhard, 1980). Embryos born from mothers lacking a functional copy of one of these genes die as hollow tubes of dorsal tissue. "In contrast to *bcd,* there is no evidence for a localization of the transcripts of any of these genes in the egg, suggesting that D/V polarity is generated by a mechanism different from that organizing A/P polarity" (Ingham, 1988). These functions have been mainly studied by experiments involving the rescue of mutant embryos by transplantation of wild-type cytoplasm or RNA (Anderson, 1987). The best candidate for a gene encoding such a product is *dorsal (dl).* This maternally active gene directs the progressive localization of *dorsal*$^{+}$ activity to ventral regions of early embryos.

FIG. 35.

Polar axiation in Diptera.

(A) Sequence of initial events of bipolarization in the eggs of *Drosophila:* (1) *bicoid (bcd)* coded-proteins localized at the anterior pole; *nanos (nos)* gene products localized at the posterior pole. (2) graded distribution of the *bcd* protein. *Hunchback (hb)* gene activated while cleavage nuclei have reached the posterior periphery of the developing embryo. Adapted from Ingham, 1988.

(B) Polarity deviation of *Drosophila* embryos mutationally-provoked: wt = wild type; *bcd* = *bicoid,* lacking head and thorax; *os* = *oskar,* lacking abdomen; *tor* = *torso,* lacking head and end of abdomen. Adapted from G. North, Nature, 332: 785, 1988.

(C) Embryological polarity (anterior and posterior indicator proteins) in the eggs of the midge fly *Smittia* disturbed by centrifugation (CE) resulted in double cephalon, by UV-irradiation resulting in double abdomen. In the middle, control eggs. Adapted from Kalthoff, 1984.

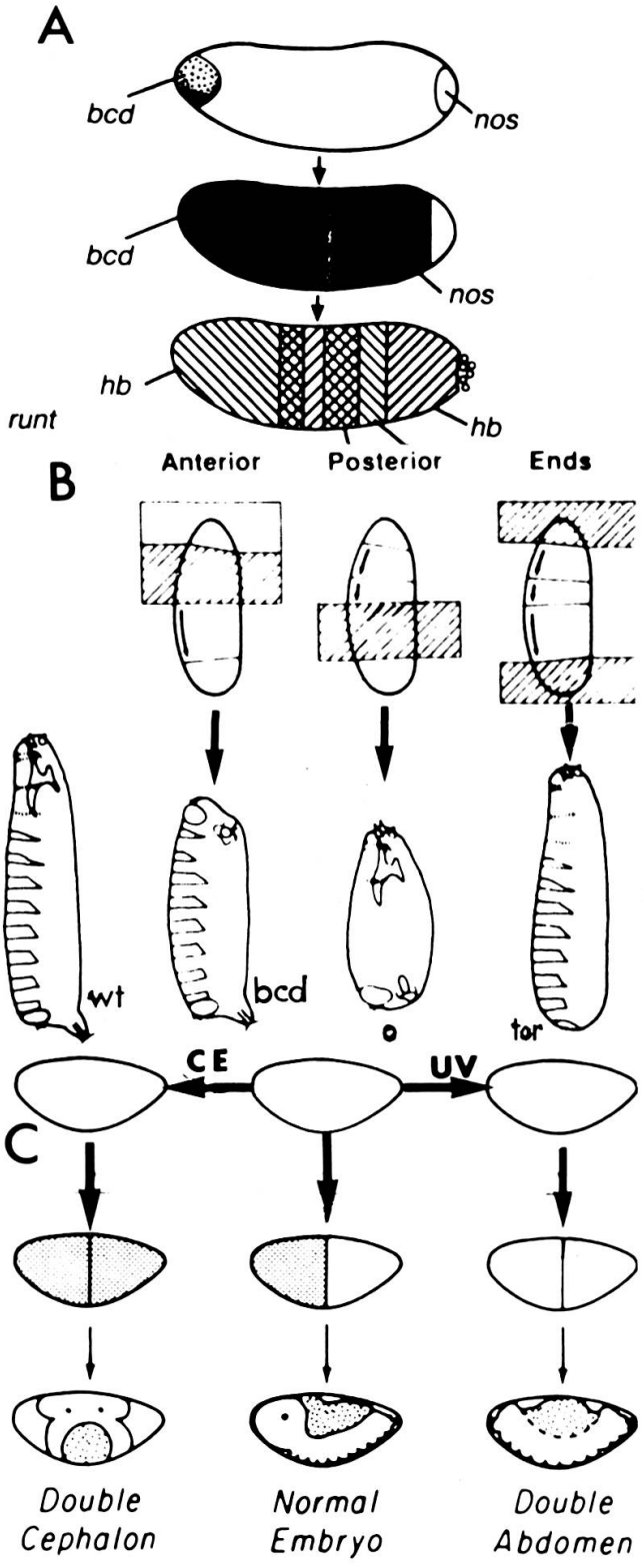

A
bcd
nos
bcd
nos
hb
runt
hb
B
Anterior
Posterior
Ends
wt
bcd
o
tor
CE
UV
C
Double Cephalon
Normal Embryo
Double Abdomen

FIG. 35.

Another of this D/V genes, *snake,* could be rescued by microinjection of a fragment of cloned DNA. Its encoded information is expressed in a serine protease, suggesting a role for this enzyme in the proteolytic cleavage of other spatially localized products (DeLotto and Spierer, 1986). It thus seems that the anterior body pattern of *D. melanogaster* is specified in large part by the protein product of *bcd* and *snake* genes which function as graded morphogens with their peak of expression at the anterior pole of the embryo (Driever and Nüsslein-Volhard, 1988). As a result of the preceding gene activities, when the mature egg is laid, it is both morphologically and molecularly polarized (Ingham, 1988).

The fundamental role of these gene products in pattern specification is illustrated by their mutant phenotypes. Thus, in the diencephalic mutation of *Drosophila,* nurse cells are found at both poles of the egg instead of only near the anterior pole as in a normal follicle. Bipolar follicles give rise to embryos having two sets of anterior structures (two-headed monsters) but altogether lacking posterior structures.

As commented by Gehring, in 1985, "observations of such maternal-effect mutants suggest that the egg cytoplasm contains substances that define the spatial coordinates of the future embryo. After fertilization, when the nuclei migrate to the egg cortex, they encounter these substances and become committed to particular fates according to their position in the cortical cytoplasm. Little is currently known, however, about the substances coded for by the maternal genes that endow the egg cytoplasm with its spatial polarity". There is only some recent evidence that maternal messenger RNA (mRNA) stored in the egg has a role in specifying the dorso-ventral polarity (Nüsslein-Volhard *et al.,* 1987).

Segmentation mutations

Embryological and genetic experiments provide compelling evidence that the segment pattern of the insect body is specified by a dynamic system of spatial cues generated at the blastoderm stage of embryogenesis. Each of the genes providing these spatial cues is defined by characteristic deletions of segments in embryos in which the gene is mutated. Genetic analysis has allowed *three* classes of zygotic segmentation genes, which relate to three different levels of spatial organization during segmentation, to be distinguished: gap genes act on the entire egg as a developmental unit, pair-rule genes act on double segmental units and segment polarity genes at the single segment level.

In the early *Drosophila* embryo, the transcripts of several pair-rule genes accumulate in a series of rapidly evolving and partially overlapping stripes (parasegments) along the antero-posterior body axis (Hafen *et al.,* 1984). During the late cellular blastoderm stage of *Drosophila* embryogenesis, the segmentation genes *engrailed (en)* and *wingless (wg)* become expressed in two series of 14 stripes which will subsequently coincide with the anterior and posterior limits of each parasegment

(Ingham *et al.*, 1988). Homoebox-containing pair-rule genes such as *fushi tarazu* (Japanese for "not enough segments") and *eve* which encode homeodomain proteins have been found to act as regulators of the segmentation genes: positively for *engrailed* controlling the pattern of stripes, negatively for *wingless* (Ingham *et al.*, 1988).

Asymmetrically distributed *dorsal*$^+$ products may play a key role in establishing diverse patterns of zygotic gene expression, and the differentiation of D/V pattern elements (Levine, 1988). For example, one of the crucial steps in the differentiation of the segmentation pattern along the A/P axis is the localization of maternally-encoded *bicoid (bcd)* transcripts to the anterior pole of the developing oocyte (Frohnhöfer and Nüsslein-Volhard, 1986; Frigerio *et al.*, 1986), which appears to be facilitated by an inherent A/P polarity within the *Drosophila* egg chamber.

Homeotic mutations

These dramatic derangements of *Drosophila* development could not be better described than by their most skillful hunter, Walter Gehring (1976), who has been fascinated by such mutations since 1965 when he described the mutant "Nasobemia" that had legs on its head in place of antennae. Previously found mutations in the homeotic gene *aristapaedia* also lead to antennal stuctures being replaced by leg ones (Pasteels, 1951).

The genes first isolated from homeotic mutants of *Drosophila* were defined as the homeobox (Gehring, 1984, 1987). They have DNA sequences in common which code for a protein that, by its functional segment or homeo domain, binds to DNA and, thereby, regulates all the genes needed to control insect morphogenesis.

The developmental pathways for many of the body segments (third thoracic and abdominals) are specified by a cluster of homeotic genes, the so-called *bithorax* (BX) complex (Bender *et al.*, 1983). Both left and right halves of this complex have been explored by chromosomal walks. Discrete genetic units are sequentially activated in each abdominal parasegment. Intricate patterns of BX expression result from the complex *cis*-regulation and the *trans*-interactions between homeotic genes that they mediate (Peifer *et al.*, 1987).

Another cluster of homeotic genes found in the *Drosophila* genome was referred to as the *antennapedia* complex and there are other mutations within the bithorax complex (Lewis' studies, see Gehring, 1985) including *bithoraxoid,* which make the first abdominal segment into a third thoracic segment. Moreover, positional information for the developing limbs is provided by a proximal-distal pattern-forming system controlled by Distal-less genes encoding a homeodomain protein (Cohen *et al.*, 1989).

d^2 *Wing patterns*

A good model system to investigate the generation of polarity pattern is the wing of *Drosophila.* It consists of a dorsal and a ventral sheet of epithelial cells (containing

± 26,000 cells per surface) that produce parallel hairs (Garcia-Bellido and Merriam, 1971). The bristles (four-cell sensory organs) and hairs (cellular extensions that later become sclerotinized) that decorate the wing cuticle have been used as an indicator of fundamental cell polarity (Nüsslein-Volhard and Wieschaus, 1980; Wieschaus and Riggleman, 1987).

The *frizzled (fz)* locus is required for the development of a parallel array of bristles and hairs on the adult cuticle of *Drosophila melanogaster* (Gubb and Garcia-Bellido, 1982). Weak *fz* alleles cause a swirling of hairs in some regions of the wing while strong alleles result in a nearly random orientation of hairs in much of its central region. In their efforts to understand how a group of cells, such as the epidermis of *Drosophila,* coordinate their cytoskeletal outgrowths to produce a parallel array, Vinson and Adler (1987) suggest that "*fz* has two mutably separate functions in establishing hair polarity on the wing. One function involves the transmittance and/or generation of a polarity signal along the proximal-distal axis of the wing. The second function involves the cellular interpretation of a polarity signal." The tissue polarity *fz* locus would encode a protein with seven putative transmembrane domains (Vinson *et al.,* 1989).

e) *Ascidian-Tunicates*

After fertilization, the ooplasm is rearranged by a spectacular episode of cytoplasmic movements known as ooplasmic segregation (Whittaker, 1979). This process begins with the myoplasm and ectoplasm along the vegetal cortex to the future posterior region of the embryo where they are extended into intensely pigmented crescent-shaped patches, the yellow crescent in *Styela* and the orange crescent in *Boltenia.* Ooplasmic segregation is concluded when the ectoplasm streams into the animal hemisphere, accompanied by the male pronucleus, and the endoplasm returns to its original position in the vegetal hemisphere.

Ooplasmic segregation and changes in the distribution of mRNA molecules are among the earliest indications of polarity in the ascidian egg. The colored ooplasmic crescent has been exploited to study this distribution of mRNA with respect to cytoplasmic localization (Whittaker, 1979).

Jaffe (1981) has suggested that a gradient of calcium ions, initiated from the point of sperm entry, polarizes the direction of ooplasmic segregation and mRNA localization in ascidian eggs. The orange crescent in the egg of the tunicate *Boltenia villosa* forms on the high side of a calcium ionophore A 23187 gradient formed with the glass fiber technique (Fig. 34 A). This pigment and mitochondrial segregation normally mark the future dorsal side of the embryo. However, when eggs are activated by the ionophore by placing it in the gradient, 82% of the activated eggs form orange crescents and mitochondrial localization with midpoints 45° or less from the point nearest the ionophore-coated fibers. Moreover, 10% of the eggs positioned

between two coated fibers form two orange crescents, each about half the normal size. This supports the hypothesis that there is *no* predeterminant polarity of cytoplasmic localization in the ascidian egg, and suggests that cytoplasmic Ca^{2+} gradients can strongly influence this axis of polarity (Jeffery, 1983).

Bipolar regeneration also occurs in very short pieces of the abdominal region of the ascidian *Clavelina* (Karp and Berrill, 1981). The anatomy of this ascidian is not important here, except that a nerve cord is not present. In this ascidian, the timing of cuts is as important as the distance between them. If short pieces are isolated by cuts made almost simultaneously, anterior (thoracic) structure forms from both surfaces. If the posterior cut is made an hour, more or less, *after* the first cut, typical anterior and posterior regeneration occurs at the two surfaces respectively; i.e., the original polarity of the tissue persists. The orientation, presumably of macromolecular components, may be more significant than concentration gradients of either ions or molecules in determining and maintaining tissue polarity. The electric DC current of injury may well be responsible for establishing the initial direction of polarization. That is, in short pieces resulting from simultaneous cuts, the polarizing current produces its effect in opposite directions from the two ends.

f) *Amphibians*

At fertilization the egg contains an animal-vegetal polarity that is interpreted morphogenetically during embryogenesis. Thus, the types of cells developing from different regions of the egg can within certain limits be predicted: "the animal cell cap provides ectoderm, neural tissue, and mesodermal cell of various kinds; the equatorial or marginal zone gives rise to a major portion of the mesoderm, as well as to other cell types; and the vegetal region provides endoderm, but also other cell types, including (in anuran amphibians) the germ cell progenitor" (Davidson, 1986). After the egg is activated by sperm penetration, the A/V axis is preserved, but its radial symmetry is superceded by the emergence of bilateral symmetry. In some anuran eggs (e.g. *Xenopus laevis*), the sperm entrance site is visible as a dark spot and conveniently marks the future ventral region (Fig. 36 A), while in several urodele eggs (e.g. *Amblystoma mexicanum*) only a surface pigmentation alteration — the gray crescent — marks the future dorsal area (Malacinski, 1984).

The dorsal-ventral axis is established initially by movements of preformed cytoplasmic consituents following fertilization, and prior to first cleavage. During cleavage the animal-vegetal and dorso-ventral axial specifications are transmitted to the blastomeres that inherit the respective regions of egg cytoplasm, and there is initiated the first of a series of inductive interactions by which the larval body plan is ultimately established (Fig. 36 B). This axis, which is set up during oogenesis, defines the future anterior-posterior polarity of the tadpole; it is thus an example of a developmental outcome predetermined by cytoplasmic localization of informa-

tion in the egg (Dawid and Sargent, 1988). The second major axis of the tadpole is fixed only after fertilization. Sperm entry can occur anywhere in the animal hemisphere and, as described above, the sperm entry point (SEP) normally defines the future ventral side. The way in which this polarity determination comes about

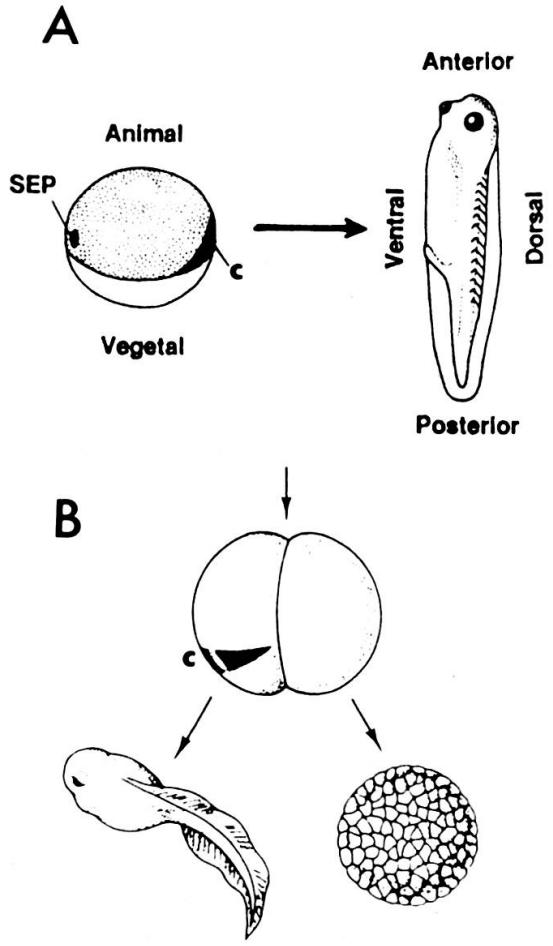


FIG. 36.

Polar axiation in developing Amphibians.

(A) Relation between the polarity of the fertilized egg and that of the tadpole. c = grey crescent; SEP = sperm entry pole. Adapted from Dawid and Sargent, 1988.

(B) Role of the grey crescent (c) in the early development of frog embryo: if the first cleavage (vertical arrow) does not pass through it, only one of the two daughter cells develops into an organized embryo. Adapted from Grant, 1978.

has been explained primarily through the work of Gerhart and colleagues (1983, 1986). A movement of cytoplasm relative to the cortex takes place between fertilization and first cleavage, and it is the direction of this movement that is the most accurate predictor of the future dorsal-ventral polarity of the embryo (Vincent *et al.*, 1986).

In normal development the vegetal pole cytoplasm or "germ plasm" is distributed to all of the first four blastomeres, and each has the capacity to generate a functional germ cell lineage. The primordial germ cells reside in the endoderm during early development (Grant, 1978).

A closer focus on the events occurring at the 8-cell stage reveals a radial intracellular polarization of the blastomere cytology, which is mediated by intracellular contact. At 4th cleavage the peripheral cytoplasmic regions are physically segregated to the outer cells of the 16-cell morula. By the end of the 8-cell stage a major cytoplasmic reorganization has occurred (reviewed in Johnson and Pratt, 1983; Johnson *et al.,* 1984). The peripheral cytoplasm of the polarized 8-cell stage blastomere acts as a determinant for trophectoderm differentiation, while the cells inheriting the internal cytoplasm are destined to become ICM cells, i.e., unless they are placed in circumstances where they are induced to polarize anew. The overall consequence of this polarization is a change in form of the embryo from a loose aggregate of spherical cells to a tightly compacted, radially organized embryo.

Polarization is an inductive process which requires intercellular contact. Furthermore, continued cell contact is needed to maintain the state of polarization initially imposed. When cultured *in vitro,* polar 16-cell blastomeres display trophectoderm-like behaviour, and they wrap around nonpolar cells, while apolar cells cultured together tend to polarize, and to produce aggregates including both external trophectoderm-like cells, and inner, ICM-like cells (Johnson and Pratt, 1983).

Both the cytoskeleton and gravity act in the determination of the dorso-ventral polarity (Ubbels and Brom, 1984). Application of centrifugal forces (Dalcq and Pasteels, 1937) can overrule the strength of the cytoskeletal elements and reverse D/V polarity even shortly before first cleavage. Such experiments strongly argue for a role of gravity in the determination of embryonic polarity.

A secondary dorso-ventral RNA gradient superimposes itself on the primary polarity gradient. As a result of further synthesis and of morphogenetic movements, antero-posterior (cephalo-caudal) and dorso-ventral RNA gradients are present in the neurula and young tadpole. These gradients parallel the well-known "morphogenetic gradients" of experimental embryologists (Brachet, 1967). If, as we have assumed, a dorso-ventral gradient of messenger RNA production exists at the gastrula stage, cephalo-caudal and dorso-ventral gradients in the distribution of polyribosomes, and hence of protein-synthesizing ability, would necessarily be formed in gastrulae and neurulae. The existence of dorso-ventral and animal-vegetal gradients can also be demonstrated in studies on the reduction of vital dyes. Thus dorso-ventral reduction gradients are very apparent in early and late gastrulae and the vegetal pole shows the least activity. The parallelism between the amphibians and the echinoderms (VIII.B.2c) makes it likely that gradients in the distribution of mitochondria also exist in amphibian eggs: a dorso-ventral gradient would

superimpose itself, at gastrulation, on the initial animal-vegetal gradient (Brachet, 1957).

g) *Fishes*

In many shark genera, teeth shapes are polarized, and their cusps are deflected in a lateral-posterior direction parallel to the jaw margin. Developmental studies suggest that polarity and tooth shape are regulated independently as shown by experiments of injury induced polarity reversals (Reif, 1984).

h) *Birds*

On the vitelline surface of birds' eggs, the primitive embryonic streak develops a thickening, the Hensens's node. As the node retreats, the body axis is taking shape anterior to it. Thus, the formation of the anterior end of the body is proceeding well before gastrulation has been completed posteriorly. The polarity of the avian embryo is thus evident prior to gastrulation, during the formation of the area pellucida. The thinning of the area pellucida begins at the presumptive posterior end, where the aggregation of cells to form the hypoblast also begins. This end of the area pellucida retains its organizational capacity at gastrulation as well. However, if the entire area pellucida of an embryo is rotated 180°, the embryo develops with reversed polarity while if a central square — devoid of the posterior margin — is cut out of the area pellucida and rotated, polarity is unaffected. These experiments conducted by Spratt and Hass (1960) provided evidence of the production by the posterior zone of the area pellucida of a morphogenetic factor organizing polar axiation of the avian embryo (Fig. 37 B_1).

The determination of bilateral symmetry of chick blastodiscs, earlier thought to be caused by the rotation of the egg in the mother's uterus, is, in fact, a result of the force of gravity. Axial symmetry of the blastodisc can be changed by altering its spatial position and axis determination shown to be a gradual phenomenon correlated with the morphogenetic process of formation of the area pellucida (Eyal-Giladi and Fabian, 1980).

The polarizing region, a small group of cells at the posterior margin of the avian limb bud, acts as a signalling region to specify the pattern of structures which develop across the antero-posterior axis of the limb. There is indirect evidence that the signal from the polarizing region is a diffusible morphogen (Tickle, 1980). The effects of multiple polarizing region grafts have also shown a positional signalling along the antero-posterior axis of the chick wing (Wolpert and Hornbruch, 1981).

i) *Mammals*

In the mouse, the earliest signs of contact-induced polarization in blastomeres might occur as early as the four-cell stage but throughout this period the polarity that is induced is labile (Johnson and Ziomek, 1981*a, b*).

The induction of polarity in the eight-cell mouse blastomere takes between three and five hours and after this period the axis of polarity is stable. Prior to this, however, a change in the location of the inducing signal will result in a predictable change in the final axis of polarity achieved (Johnson and Ziomek, 1981*a, b*). The axis of polarity can be controlled by the geometry of the contacts (Fig. 37 A): the apical pole always forms at a point opposite the contact, and in the case of multiple contacts, at a point equidistant from them.

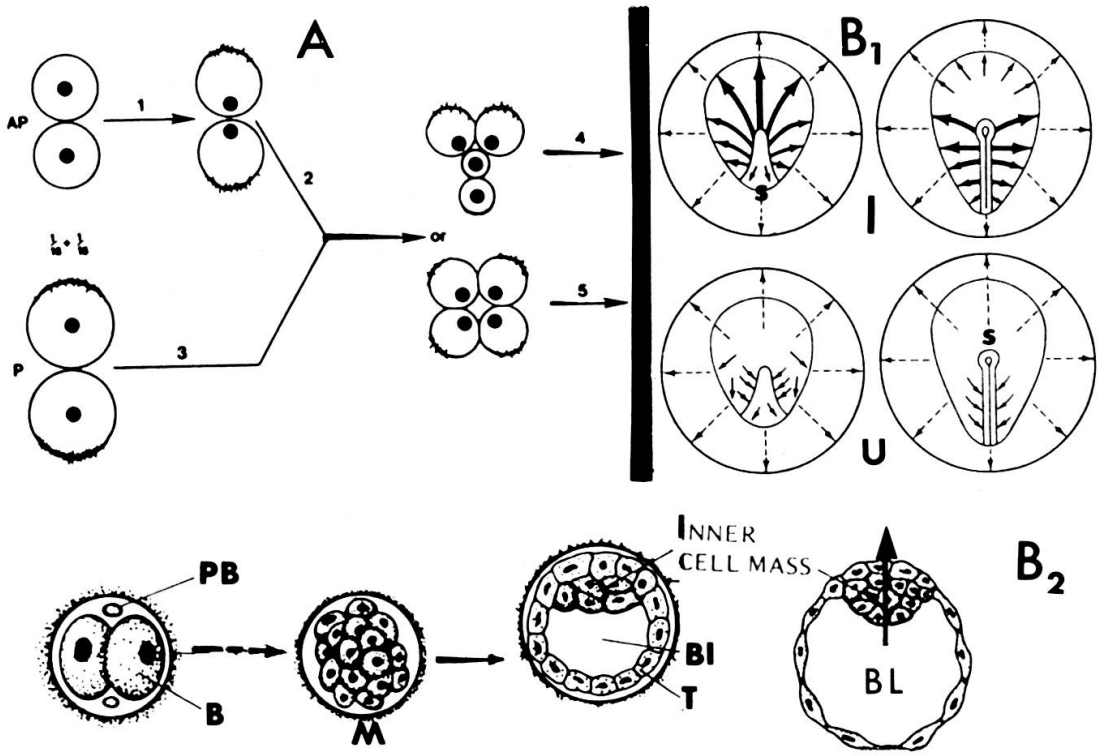


FIG. 37.

Polarity in Birds and Mammals.

(A) Induction of polarity in two apolar cells due to their asymmetry of contacts (1) at early embryogenesis in mouse. A couplet of polar cells then behaves in one of two ways: both cells may divide parallel (3) to the axis of polarity; when they divide normal (2) to the axis of polarity each of them generates one apolar and one polar cell. Adapted from Johnson and Pratt, in Jeffery and Raff, 1983.

(B_1) Polarized movements of embryonic layers in the expanding blastoderm of chick. The thickened arrows from the anterior portion of the primitive streak(s) mark the ingressing cells of presumptive endoderm and mesoderm. Lower (l) and upper (u) surfaces. From Spratt and Hass, 1960, in Grant, 1978.

(B_2) Early human embryogenesis: from the first holoblastic cleavage, through the relatively apolar morula (M) arises a blastocyst (Bl) anterio-posteriorly polarized (arrow). B = blastomere; PB = polar body; ICM = inner cell mass; T = trophoblast. After Loomis, 1986.

During the life of each eight-cell blastomere, the surface of the cell is reorganized fundamentally to yield a polar phenotype with an outward apical pole of microvillous membrane and an inner basolateral membrane largely devoid of microvilli (Johnson and Pratt, 1983). This polarized surface phenotype assumed by each eight-cell blastomere is stable and persists after isolation of single cells and during the division of eight-cell blastomeres to the 16-cell stage either in isolation or in situ (Johnson and Ziomek, 1981*b*; Reeve, 1981).

In conclusion, contacts with competent inducing cells appear to account for polarization of rodent embryos while induction of polarity does not require the formation of intercellular junctional complexes (ionic media, antisera, and drugs that block junction formation do not prevent polarization, Johnson *et al.*, 1984). However, remain the questions rised by Johnson and Pratt (1983) "Since developmental localization within the mouse embryo first becomes stabilized at the eight-cell stage, does this mean that the cell interactions leading to polarization occur under embryonic rather than maternal control? This question cannot yet be answered unambiguously. However, it does seem likely that transcriptional activity is not required during the four-cell and eight-cell stages."

In man, it is only at the postimplantation period of a blastocyst to the uterine wall that the embryonic A/P axis is established (Fig. 37 B_2). Microvilli in the blastomeres surface contain bundles of contractile microfilaments which may function in bringing together the surfaces of adjoining embryo cells. Certain microvilli are localized on the apical surface of these blastomeres, giving each cell a distinct polarity. It is the apposition of the adjoining surfaces which leads to the formation of desmosomes, gap junctions, and tight junctions between the cells of the embryo, which are believed to have important structural and developmental roles (Karp and Berrill, 1981).

3) Triaxial patterns (A/P + D/V + Left-Right (L/R) polarities)

a) *Helical bacteria*

Many types of bacteria are helical. Of the 17 identified species of the Aquaspirilla, twelve are right-handed and three are left (two are straight). It is claimed that within a species all individuals have the same hand.

A temperature-induced switch of hand in the highly organized multicellular structure of *Bacillus subtilis* "macrofibres" has also been observed and studied in some detail (Favre *et al.*, 1985). But are either of these inversions (reflections) genetic in origin?

b) *Green plants*

Cell division of the apical cell of root on the right-handed side of the water fern, *Azolla,* is clockwise (right-handed); similarly, in root of the left-hand side of the plant, division of the apical cell proceeds anticlockwise (Gunning *et al.,* 1978).

c) *Protozoa*

Mirror-imaging by reversal of right-left asymmetry has been observed in many Protozoa. Mirror-image patterns can arise without microsurgically generated polar reversals. The clearest cases have been observed in *Blepharisma* (related to *Stentor*) and in *Tetrahymena.* There are two such situations, one involving a usual pattern juxtaposition in wild-type cell, the other the action of a mutant gene. In the *janus* symmetry-reversal mutant, the ciliary rows of such cells are always normally oriented and never inverted, indicating that there has been an *in situ* reversal of right-left asymmetry without any preceding folding or rotation of cell parts (Frankel, 1984).

A considerable body of circumstantial evidence supports the idea that water temperature has determined the hand of some Foraminifera tests. In *Globigerina* spp., left-handedness is associated with low and right-handedness with higher temperatures (Galloway, 1987).

d) *Worms*

The crown of the sabellid worm is bilaterally distinct, the right and left halves developing independently of each other (see Karp and Berrill, 1981).

e) *Molluscs*

A classical example, discovered half century ago, is that in the mollusc shells the chirality of the spiral cleavage is controlled by maternal genes expressed during oogenesis. Indeed, dextral cleavage depends on a product of the dominant gene (Freeman, 1983). The freshwater snail, *Lymnaea peregra,* has either right-handed (dextral) or left-handed (sinistral) twists to its shells. The patterns of cleavage in *Lymnaea* embryos is also spiral, and even at the first division a handedness can be seen. They cleave in either a dextral or sinistral manner. By the third cleavage the asymmetry is easily observed. The decision as to right- or left-hand cleavage that ultimately results in snails with differently twisted shells is determined by a single gene, *D.* Mutations in this gene result in left-handed cleavage that leads to left-handed snails. The product of the *D* gene must be present in the egg before fertilization for dextral cleavage can occur (Freeman and Lundelius, 1982).

At both the animal and vegetal poles of the red or green colored eggs of the elephant tusk molluscs, *Dentalium,* there are unpigmented regions of cytoplasm relatively free of yolk. The first few cleavages of *Dentalium* are markedly asymmetric. Following fertilization, the bulk of the yolk-free cytoplasm at the vegetal pole is blebbed out into a lobe (Loomis, 1986). Animal-vegetal polarity in the plasma membrane of the molluscan egg has been studied by quantitative freeze-fracture (Speksnijder *et al.,* 1985). The yolk-free material in the polar lobe is essential for differentiation of the mesoderm rudiment in the larva. If the polar lobe is nipped off at the time of either the first or second cleavage, the larva that develops lacks the mesoderm rudiment.

Steric constraints apparently lie behind molecular processes and if they permit one hand, almost without exception, they forbid the other. Could steric considerations similarly explain the preference of right- over left-handed conchospirals? Perhaps surprisingly, the answer may well be yes — at least in part — surprisingly because the steric considerations in this instance are behavioural (Galloway, 1987).

How could evolution discriminate against a rare left-handed form in a predominantly right-handed population and *vice versa*? Sturtevant (1923) may have made the significant observation here. Commenting on rare left-handedness in the European pond snail *Lymnaea peregra,* he pointed out that the left- and right-handed forms might not be able to mate with each other, not having a compatible screw sense! Handedness in snails is a simple mendelian characteristics (although with the interesting feature that it is an example of maternal inheritance — the hand of coiling is determined not by the individual snail's own genes but by those of its mother). Presumably an effect of the left- and right-handed forms not mating with each other would be to create separate breeding populations.

In *Lymnaea,* a trait inherited maternally should be seen in all the offspring from the same hatching. Thus, any broods of snails should be exclusively left- or right-handed. In practice, some broods possess a few snails of the opposing hand, and in sinistral broods the mutation rate to dextrality is quite high. Freeman and Lundelius (1982) have proposed a genetic model for this phenomenon. The power of their model — or alternatives to it — to explain predominant right-handedness needs exploring.

What is now known from studying *Lymnaea* is that the dextral gene is expressed during oogenesis and its product — whatever it is — controls the symmetry of the pattern of very early cleavage. An injection of cytoplasm from dextral eggs reverses the early cleavage pattern of sinistral eggs, but one from sinistral eggs does not influence dextral ones. To resolve this apparent paradox, Galloway (1987) suggested that the normal form of *L. peregra* is actually left-handed, switchable to right-handed by the protein product of the dextral gene. Freeman and Lundelius (1982) speculate that what they have found in *Lymnaea* would be found exactly reversed in a left-handed species like *L. suturalis.* Thus the path from gene locus to cleavage pattern is likely to be far from straight-forward.

f) *Insects*

In insects, the transverse aspect of embryonic pattern formation becomes apparent in the subdivision of the germ "anlage" blastema into the left and right half, with the various structures or organ rudiments arranged from medial to lateral in mirror image symmetry on either side of the midline. Data indicate that bilateralization and transverse patterning can occur fairly late in development, and are systemic properties of the embryonic blastema rather than of the egg cell, despite the bilaterally symmetrical shape of the latter and its shell (Sander, 1984).

g) *Amphibians*

The characteristic left-right asymmetry of the definitive embryo results from slight differences in mesoderm-inducing capacity between the lateral portions of the yolk mass (Nieuwkoop, 1977). Artificial inversion of eggs has also been known for some time to result in the production of double embryos with two blastopores (Pasteels, 1938), and it has recently been shown that the same can occur even at the 2-cell stage if embryos are centrifuged at 30-50 g towards the ventral side (Gerhart *et al.*, 1981).

h) *Mammals*

It is of common knowledge that embryogeny normally directs some way the liver to the right half of the body while the heart is positioned on the left, in an equitable crosslink with the right brain hemisphere, the seat of affectivity and intuition!

EPILOGUE

However attractive in its aesthetic simplicity, the principle of electric charge symmetry, first suggested by the Yin-Yang of the oriental philosophies, and confirmed by the discovery of the primordial couple electron-positron of the nuclear physics, it could not have provided the fundament for the expansion of the inert matter of the universe and its evolution into living matter requiring differential growth along polar axes.

Polar expansion of any structure, inert (crystals, etc.) or living (cytoplasm, etc.), into an organized pattern necessarily had to involve *separation* of the two opposite electric charges. This could only be achieved by the secondary couple *electron-proton,* still charge-symmetrical but mass-asymmetrical, thereby leading to orbital spinning of the light electron around the much heavier proton; both were associated into the primeval, dipolar atom of hydrogen which can thus be considered as the basic "brick" (protium) of inert as well as living matter. The probabilistic spatial asymmetry of its electric charges was further amplified by excited ionization of H to $H^+ + e^-$. Charge asymmetry of H and of its evolving, heavier atoms (He — Be — Li — ... C — N — O — ... Fe — ...) eventually led to their association into dipolar molecules such as the life-sustainer H_2O or the NH_3, and then to the polarized biogenic macromolecules needed to orient growth of the living matter. This necessary rooting of biomolecules in the fundamental electric bipolarity could be a utilitarian answer to the statement enunciated in 1969 by the philosophically-minded nuclear physicist A. Weisskopf that "we cannot really claim an understanding of molecular processes before we know why the world consists of positively (nucleons) and negatively (electrons)-charged particles".

All cells can be considered as endowed with an intrinsic polarity. Thus there should be no fully *a*polar cells; even resting, relatively isometric cells present some degree of polarity along all their radial axes of peripheral growth. Isometrically-expanding cells present a *radial* polarity which is the sum of all bipolarizing events along bipolar (center to periphery) cell axes. This radial polarity is first expressed by the more or less central location of the nucleus and the polarity of its surrounding cytoplasm reenforced by the array of microtubules centrifugally oriented toward the cell periphery.

Polarity is most conspicuous in *mono*polarly, anisometrically growing structures such as hyphae or neurites which require processes of monopolar dominance paradoxally insured by maximally efficient, self-sustained processes of apical internal bipolarization. Any impediment of these processes by deficient energy replenishment of the electric loops or disturbed cytoskeletal integrity, etc. will damp this monopolar dominance and, consequently, allow uncontrolled *bi-* or *multi*polar growth. In agree-

ment with Bünning's axiom (1952) "Keine Differenzierung ohne Polarität", cell differentiation also depends upon internal bipolarization of an apparently homogeneous cytoplasm. It is *mono*polar when bipolarization results in only the apical pole being differentiated while it is *bi*polar when the process separates, by unequal division, two reciprocally differentiated poles. The nature of primary events is particularly critical in such stages of development, where only one or a few cell divisions must determine multiple binary choices from a single pool of genes and cytoplasm. The structure of the DNA molecule might provide a mean for distinguishing progeny cells because each daughter cell appears to receive a different complementary strand of parental DNA in each chromosome (Klar, 1987). This achievement would thus answer Sinnott's (1960) question "how far is polar behaviour an inherited character, potentially present from the beginning of development, and how far induced by the actions of various environmental factors?", a question which has also received another promising answer with the recent discovery of the selective localization of maternal messenger RNAs related to the axial polarity of animal oocytes. Remains, however, the central question of "what is the nature of the primary asymmetrical event?" *Can it simply be a sophisticated molecular extrapolation of the original charge separation proton/electron?*

At another question raised by Sinnott about polar behaviour "is polarity an aspect of the whole organism or do individual cells possess it?", it could be answered that the overall polarity of developmental patterns simply results from the superposition of their individual cell polarities. However, at the highest level of intercellular polarity, morphogenesis, the intrinsic polarity of individual cells appears to be superceded by gradients of morphogenetic substances or morphogens, generated by either simple diffusion or by polarized transport — back diffusion mechanisms, imprinting a general polarity over the field of cells (Meinhardt, 1982). The question remains, however, whether polarity and the field properties of living organisms are reductible to those of physics, or whether they are irreductible and exhibit their own distinctive phenomenology. In 1976, B. C. Goodwin's belief was that "one must remain agnostic on this until there is sufficient evidence to provide an informed conjecture". Since that epistemological statement, there is no doubt that the conceptual barrier between the living and the non-living world has continually eroded. The penetration of the concepts of physics in biology has been facilitated by the introduction of new, sophisticated instruments and techniques which have allowed a deeper study of the several functions of cell, now ultimately understood by means of simple physical concepts such as diffusion, electrical conduction, tunnelling through a potential barrier, and ... electric dipolar moments of biomolecules. The increasing success of this mechanistic world view, in which fundamental electric biopolarity, thermodynamics and quantum mechanics play a central role was already well recorded, in 1970, by Jacques Monod in his both highly rational and intuitive overview of biology "le Hasard et la Nécessité".

It is to provide the incentive for further answering these fundamental questions that we have polarized our interest on an integration of all possible knowledge about general polarity, from its physico-chemical fundaments to its multifaceted biological expression. To effect this long intellectual travel strongly appealed to us when reaching ourselves the so-called reflective stage of life. Now that it is completed, it does not leave us with the feeling that "finishing the book does mean the life" and, "in cha' Allah", we therefore expect to continue for a time to keep up with the rapidly advancing front of biopolarity, to further convince newcomers in Biology to climb the evolutive ladder from its primeval dipoles to that most thinking "vertical dipole", the human being. As "thinking head", we hope to have captured at least a spark out of the dynamic spirit of Polarity which pervades all aspects of Nature and lies at the origins of Life.

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