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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).

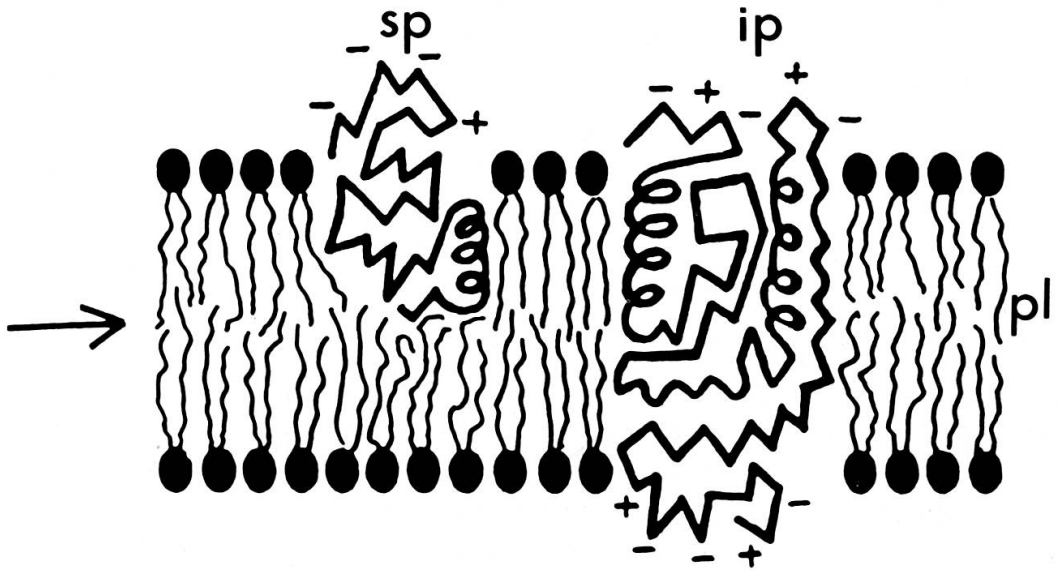

FIG. 8.

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:

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

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

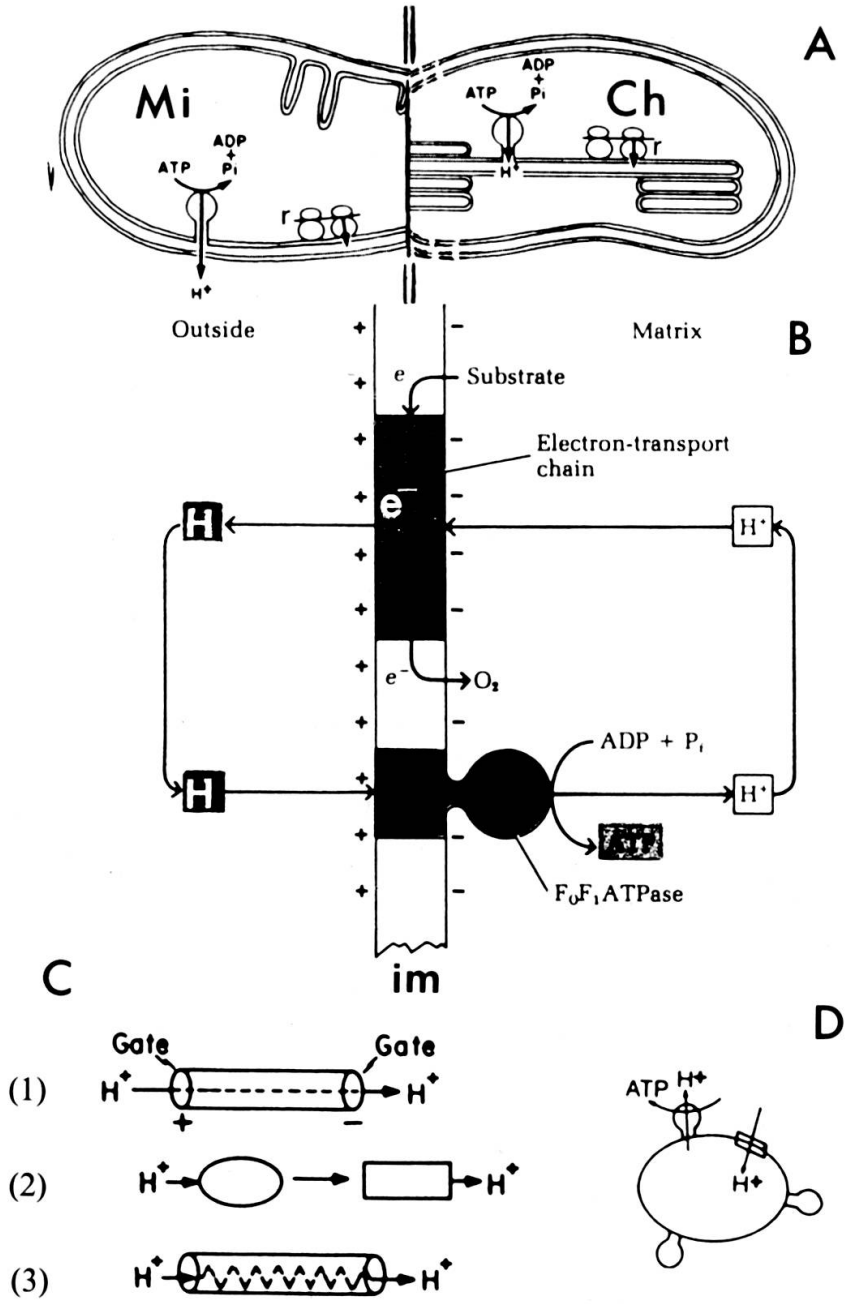

FIG. 9.

(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).

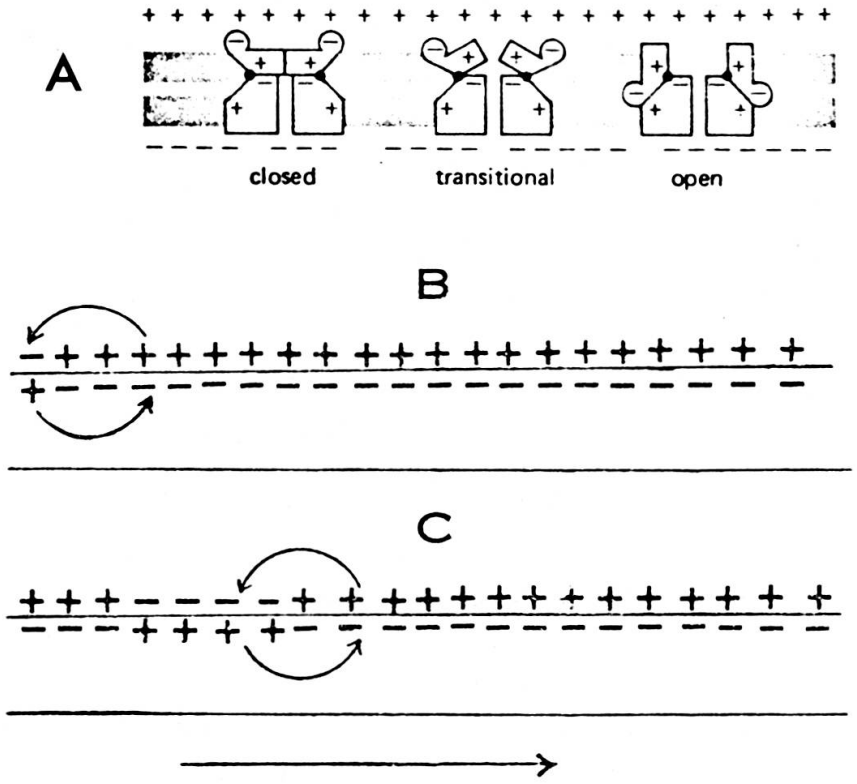

FIG. 10.

(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).

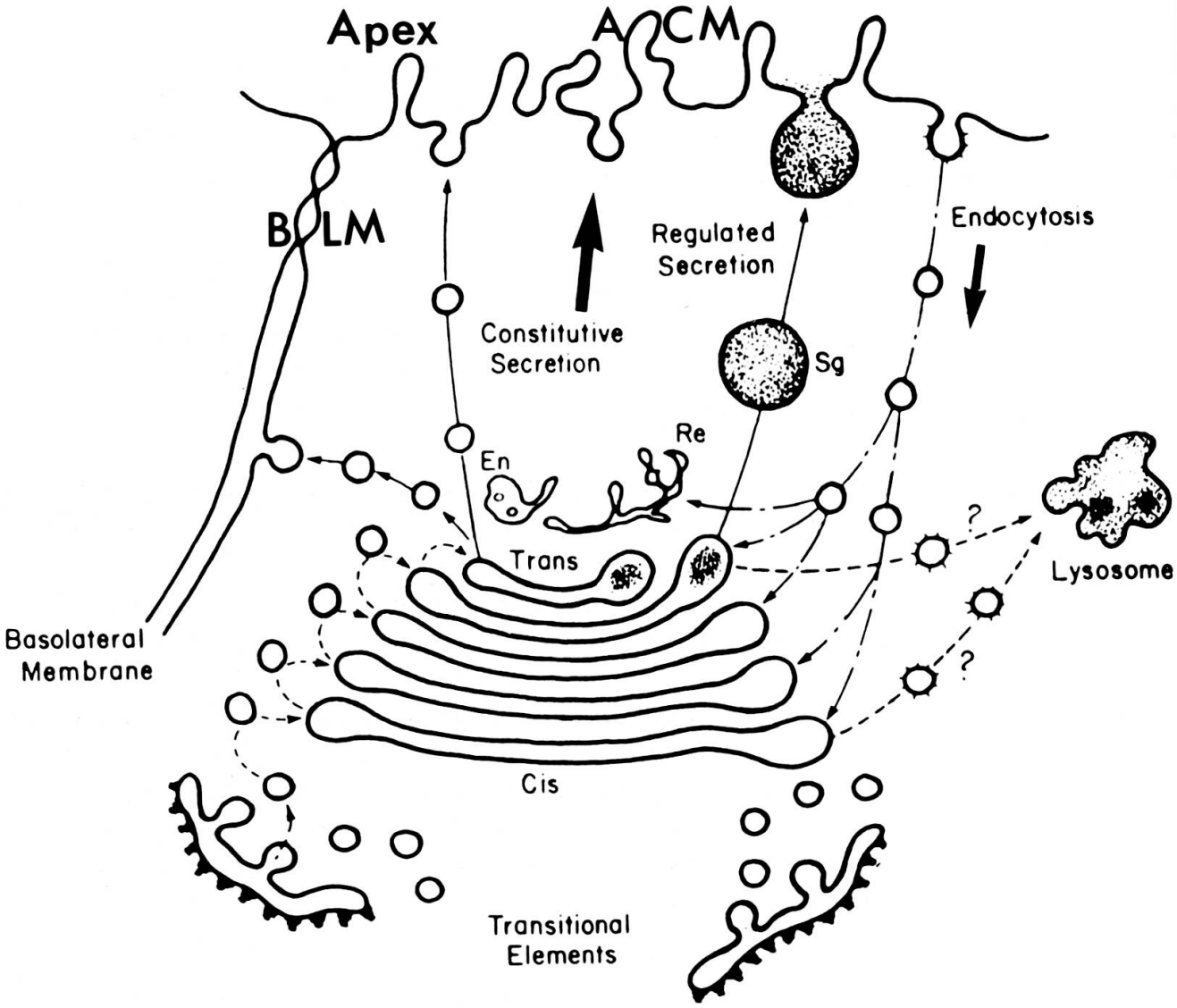

FIG. 11.

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

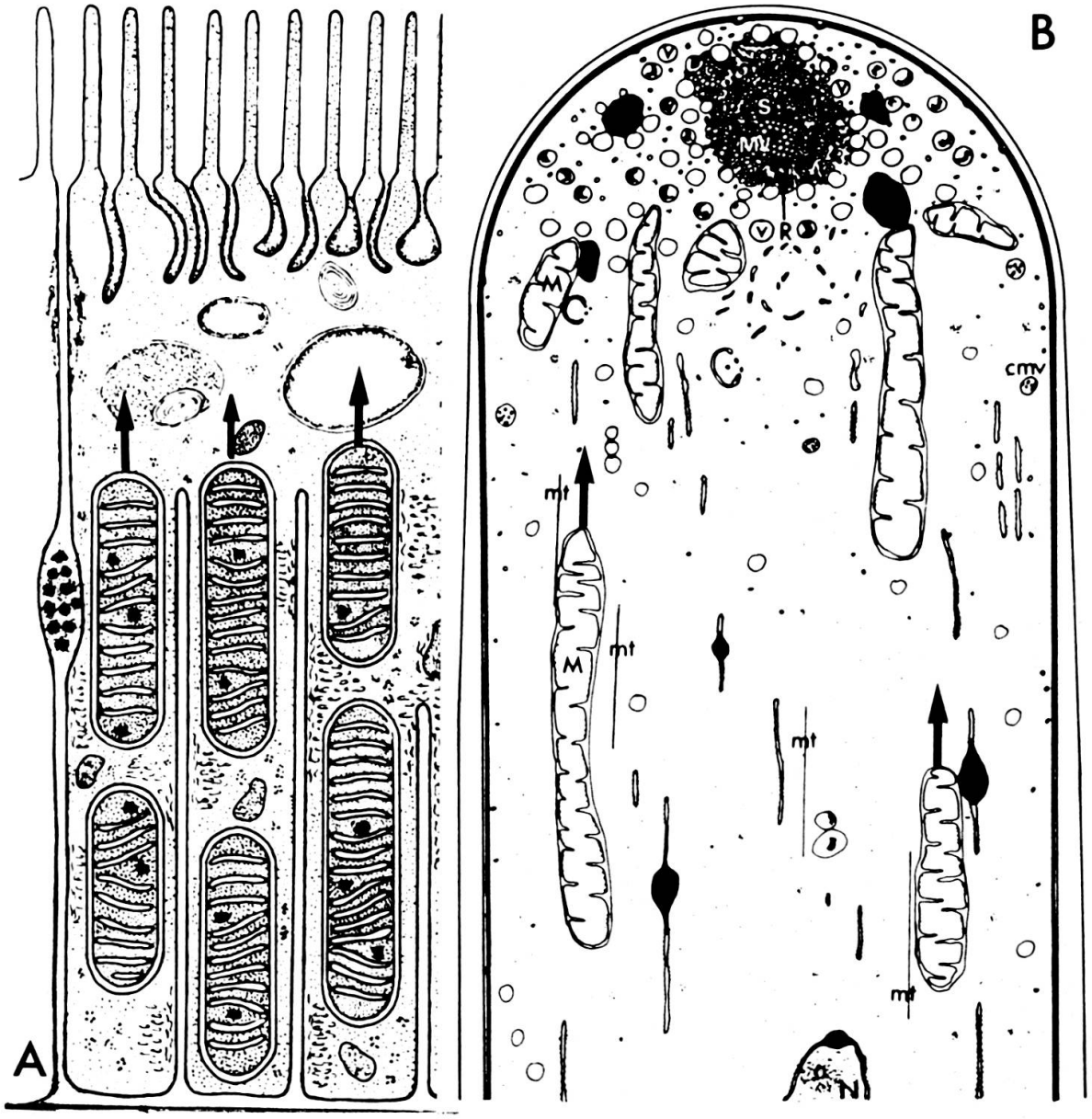

FIG. 12.

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).

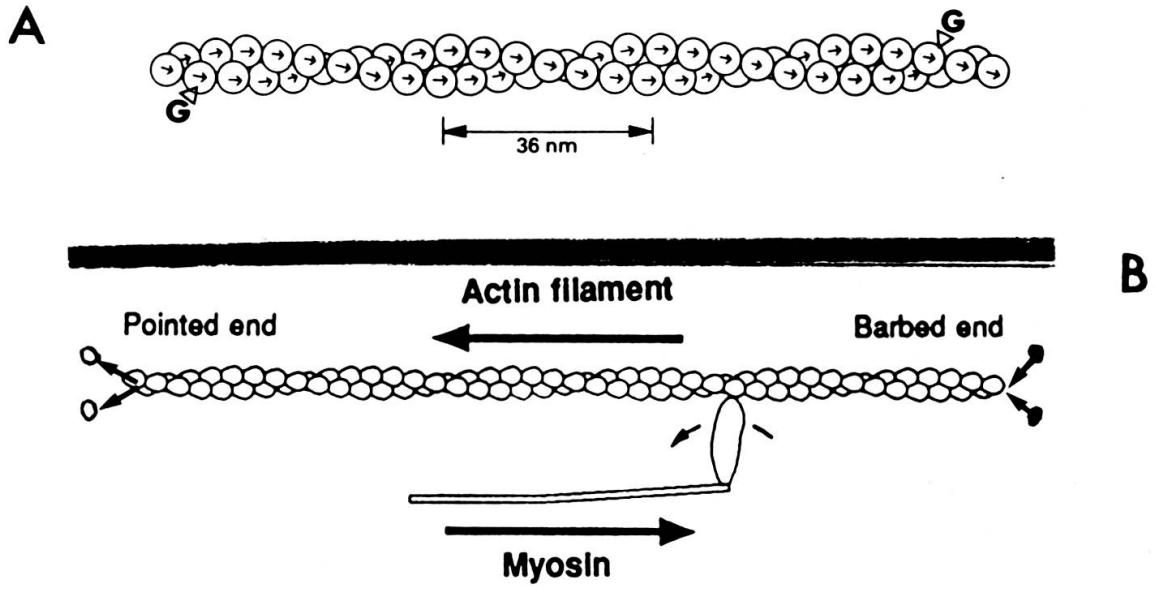

FIG. 13.

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

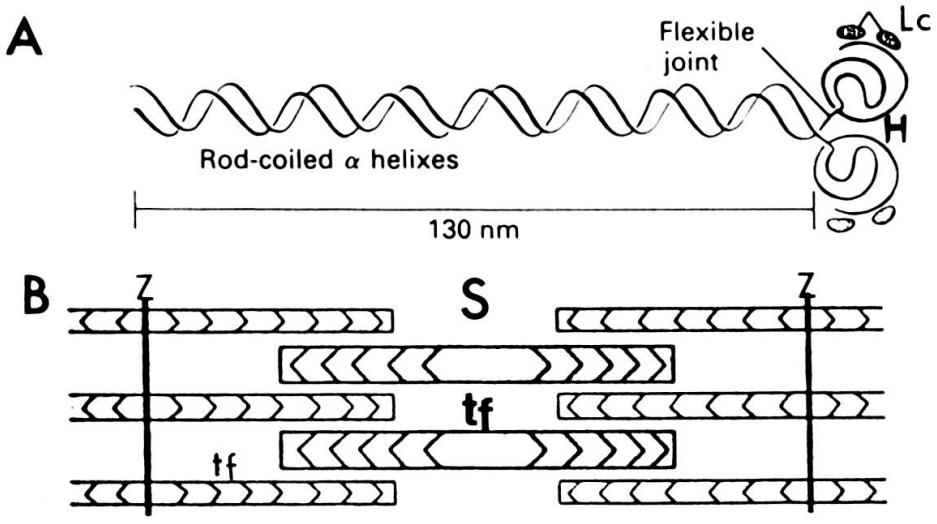

FIG. 14.

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

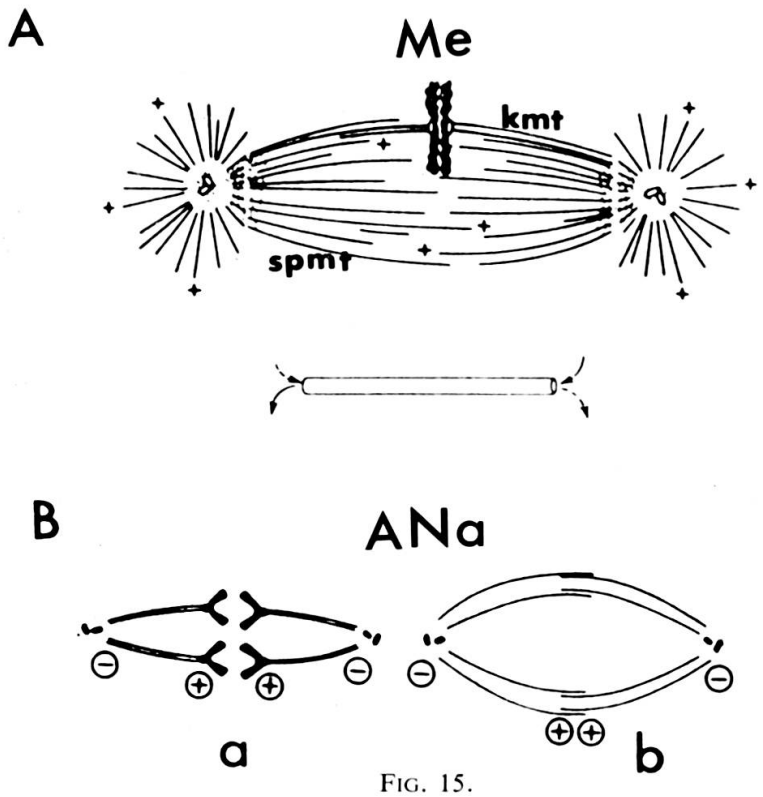

FIG. 15.

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

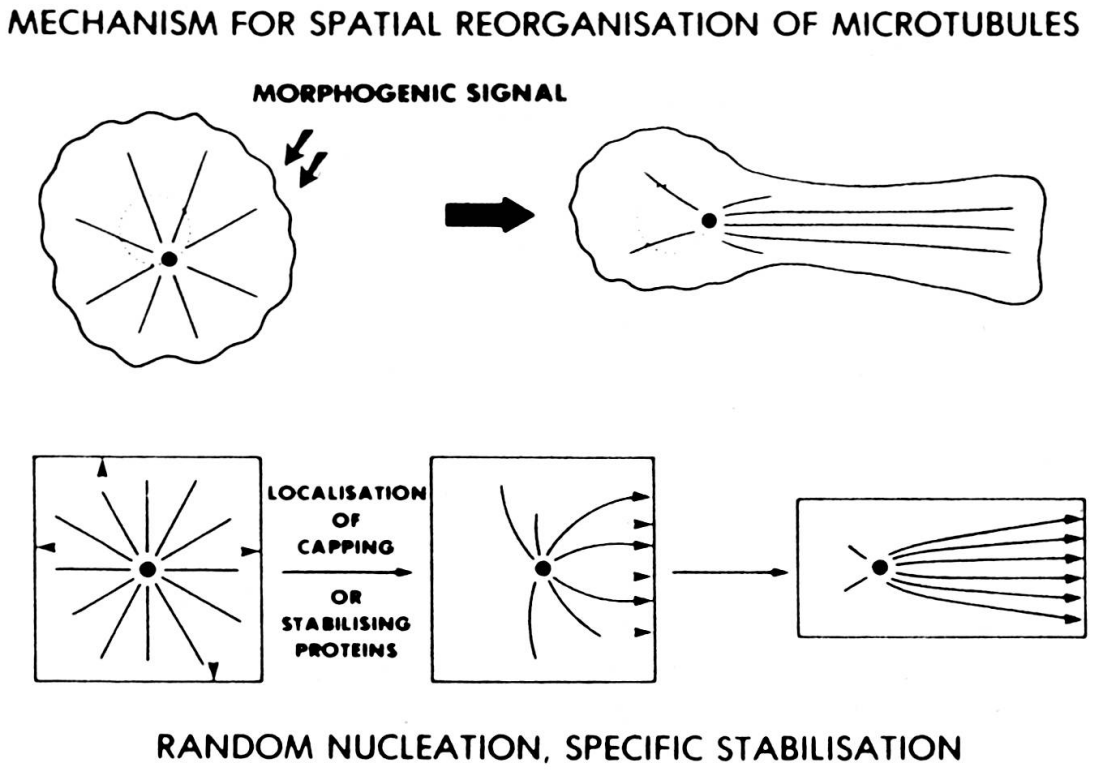

FIG. 16.

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).