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Autor: Turian, Gilbert
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VII. POLARIZED CELL DIFFERENTIATION

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

Environments can interact with genetic mechanisms to direct the accumulation of specific cellular components to precise locations within the cell. Subsequent segregation of nuclei into these polar regions of cytoplasm results in different microenvironments for each of the genetically identical nuclei. The ensuing nucleoplasmic interactions have been shown in both plant and animal cells to be the basis for selective gene expression in different regions of cells, the developing embryo, and the mature tissues. Thus the divergence of gene expression in different cells of mature tissue is initiated by the unequal distribution of cytoplasmic components, in agreement with the famous Bünning's axiom (1952*a*) "there is no differentiation without polarity".

Unequal divisions are an early expression of polar differentiation. They define one side or corner of a cell relative to all others and are thus an expression of polarity at a cellular level (Sachs, 1974). The two unequal cells produced differ not only in size but also in the structure of their cytoplasm as seen in both light and electron microscopes (Palevitz and Hepler, 1974). Such asymmetric cell division could involve the self-assembly of the microtubules initiated by a high activator concentration directing the synthesis of the new cell wall closer to the activated site while a second center of microtubules self-assembly would be created at the opposite side of the cell (Meinhardt, 1984). In the self-regulation of unequal pattern through activator-inhibitor distribution in a linear field, the activator maximum process can regenerate: "with the removal of the activated tissue, the site of inhibitor production is removed, the remaining inhibitor decays and a new activator maximum can be triggered in the remnant tissue" (see Fig. 1.3, in Meinhardt, 1984). These divisions are found in all stages of plant development (Bloch, 1965) such as in apical cellular patterns of ferns, in the cambium and many meristemoid tissues as well as different specialized cells or idioblasts. Organisms behave according to their position and show differences between axis and direction.

According to Grant (1978), the directional organized forces in the cytoplasm produce three different types of cell polarity (Fig. 26 A):

(1) with cell organelles restricted to one region, the axis of the cell being visibly defined by that organelle accumulation; such polarity is eliminated after cutting the cell, for example that from a ciliated epithelium of respiratory tract. An hyphal apex with subtip accumulation of mitochondria would correspond to that type; (2) with oriented structures uniformly distributed throughout the cytoplasm, the cell axis being defined by their orientation; here, polarity is a property of the entire cell rather than of a region and the organelles align themselves in response to forces operating within cytoplasm; consequently, polarity is retained after cutting this cell which may originate from a muscle in which longitudinal myofibrils have the same polarity as that of the surrounding cytoplasm; (3) based on intracellular gradient either of organelles or of metabolic systems with peaks of activity at one end diminishing gradually toward the other. When a high point metabolism appears at one pole, with a decrease toward the opposite pole, inversely "double gradients" may appear as the yolk-pigment, reverse distribution of the frog egg where yolk is concentrated at the vegetal pole while pigment is highest at the opposite animal pole.

A. INTERCALARY DIFFERENTIATIONS

These are primitive types of differentiation which are controlled by three different kinds of mechanisms (Bonner, 1974): (1) production of substances, (2) time of appearance of certain substances, and (3) spacing of the substances that is, their localization. Bacterial spore division examplifies the *temporal* kind in which there is a change in time only, while the *spatial* one intervenes at heterocyst formation in blue green "algae" or Cyanobacteria.

1. *Bacterial endospores*

Endospores, formed inside a bacterial cell, are the resistant forms of many bacteria; they are non-dividing, dormant cells, more resistant to desiccation, heat and other adverse conditions than the vegetative organisms. While bacterial vegetative cell division is characterized by the symmetric septation, spore formation implicates an asymmetric septation. This asymmetric septation during cell division has been regarded as an ideal model system for studying the mechanism of cell differentiation (Halvorson, 1965; Aronson and Fitz-James, 1976).

Induction of endosporulation and hence the sporulation "division" is apparently coupled to the cell division cycle at an early point in DNA replication (Hitchins, 1978). When the early stages of this complex phenomenon are considered

as equivalent to a modified cell division (Hitchins and Slepecky, 1969) directs it at the problem of the control of the topological relationship between the septum and the genome in cell division. Therefore, bacterial sporulation can be viewed as a general problem of the topology and polarity of DNA segregation (Hitchins, 1978) and as such has been considered as a model system for studying cell polarity and patterning.

Every sporogenic cell is polar (Fig. 25 A) so that asymmetric septation creates cells with different composition. The stage of partial engulfment of the pre-spore cell precedes that of the completely engulfed cell compartment or forespore surrounded by mother cell cytoplasm (Freese and Heinze, 1984). The cell is irreversibly committed to continue its differentiation when forespore engulfment is completed by a double membrane. The inner membrane has the normal orientation of a membrane surrounding a cell's cytoplasm. In contrast, the outer forespore membrane resulting from the engulfment process should have opposite polarity (Freese, 1981). Consequently, compounds whose active transport depends on the membrane polarity should not be actively transported into the forespore; facilitated transport is possible only when concentration of ions is different in the two cell compartments (Freese and Heinze, 1984).

Factors such as the ionic composition of the growth medium can influence cell polarity in *Bacillus subtilis*. They have been statistically analyzed by Dunn (1980) who has shown that, within chains of four sporangia or quads, "the probability that a spore is formed distally in relation to the newest division septum is used as a measure of cell polarity; then a cell's polarity is influenced by the behaviour of its sister sporangium (i.e. sister cells do not position their spores independently), but not by its position within a quad". After having recorded the positions of spores within the sporangia of a quad, Dunn could ask the following questions: (1) Is the polarity of a cell that gives rise to a type α-sporangium different from that giving rise to one of type β? That is, is there any effect of sporangial position within a quad on polarity? (2) Are sister sporangia interdependent or do they position their spores independently? For example, is there any influence of a type α-sporangium on its sister spores type β-sporangium, or vice versa? According to Dunn (1980) there is no difference in the polarity of the sister cells that give rise to type α or type β sporangia, even though the sister cells were found to some extent to be interdependent.

2. *Cyanobacterial heterocysts*

The differentiation of a cyanobacterial vegetative cell into a specialized, nitrogen-fixing cell, called heterocyst, generally occurs by intercalary, asymmetrical division of one of a few swollen precursor cells or proheterocysts. This developmental process is both temporally and spatially controlled (Carr and Whitton, 1982). It

resembles sporulation in its requirement for the orderly expression of sets of genes; moreover, two different rearrangements occur near the nitrogen fixation (*nif*) genes (Golden and Wiest, 1988). However, the differentiation of heterocysts differs from simple sporulation by its irreversibility even upon addition of an organic nitrogen source.

Anabaena, one of a number of species of filamentous cyanobacteria, differentiates its heterocysts at regular intervals along each filament in response to deprivation of a combined nitrogen source under aerobic conditions. The vegetative-cell functions of photosynthetic oxygen evolution and CO_2 fixation are shut down; in heterocysts, they are replaced by cyclic photophosphorylation and oxidative carbon metabolism yielding ATP and reductant for the anaerobic process of nitrogen fixation, its ultimate product.

The big questions about heterocyst differentiation have been posed at the cellular and molecular levels. Concerning the former, little is known of the rules governing the selection of vegetative cells for differentiation. In an already-differentiated filament growing on N_2, the vegetative cell placed midway between two existing heterocysts is the most likely to differentiate next. That is thought to be at the lowest point in a gradient of inhibitor flowing from the heterocyst (Wolk, 1967). Glutamine is a good candidate for the inhibitor. In the case of the transition from growth on combined nitrogen to growth on N_2, much less is known about the selection of cells for differentiation. In some species the slightly smaller daughter cell produced by asymmetric division may be selected (Wilcox *et al.,* 1975). It is possible that the candidate cells have just completed chromosome replication. The factors that govern the selection of vegetative cells for differentiation, and which determine the pattern of heterocyst spacing, thus remain mostly unknown (Haselkorn *et al.,* 1978).

B. APICAL DIFFERENTIATIONS

1. *Monopolar patterns*

a) *Fungal exosporulation*

Axial polarity implying both a clear axis and a clear direction (Bloch, 1965) precedes the asymmetrical division intervening on the apices of elongated structures. When differentiation concerns uniformly only one end of the axis can we speak of apical *mono*polar differentiation. It is examplified by the differentiation of conidia or of zoosporangia on the apices of specialized hyphae, the conidiophores or the sporangiophores respectively. By contrast, when this same end is differentiated into two different structures such as male and female gametangia in the *Allomyces,* can we speak of apical *bi*polar differentiation (*bi*polar axiation).

a[1] *Conidia*

Just as the key to hyphal growth lies at the tip (VI.A.2b) comprehension of blastic and thallic conidium initiation is suggested to be linked to pivotal events which occur at the hyphal tip and lead to the formation of one or more swollen propagules. At the vegetative-differentiation transition, when hyphal tip growth is arrested, the polar distribution of organelles, with its tip zone of mitochondrial exclusion, is dissipated and the Spitzenkörper disaggregates (Najim and Turian, 1979; Cole, 1986).

The two principal types of conidium ontogeny are referred to as "blastic" and "thallic" developments. Blastic conidia differentiate apically or laterally from a fertile hypha by the blowing out and de novo growth of part of the hyphal element and are delimited from the parent hypha by basal septum. Blastic conidia commonly secede from their parent hypha by the centripetal splitting of this same basal septum, a process referred to as schizolysis. Turian (1976*b*) has demonstrated that macroconidiation in the *Monilia* state of *Neurospora crassa* can be manipulated under experimental growth conditions so that both blastic and thallic modes of conidium ontogeny occur along aerial chains arising from the same mycelium. This process corresponds to a blastoarthric type of development (Turian and Bianchi, 1972).

By contrast, in the phialidic type of development, conidia are basipetally budded from a mother cell, the phialide and, therefore, the youngest conidium is closest to the fertile apex of the phialide. Deviations in the normal, phialoconidial ontogeny of *Penicillia* and *Aspergilli* have been observed such as the mutational change from basipetal (phialidic-type) to acropetal (blastic-type) cell proliferation. The conidial chain elongation becomes acropetal, blastosporogenic in an "abacus" mutant of *Aspergillus nidulans* (Clutterbuck, 1969), and the normally basipetal differentiation of phialoconidia is blocked at nonpermissive conditions in a temperature-sensitive and osmotic-remedial mutant of *A. aureolatus* (Vujicic and Muntanjola-Cvetkovic, 1973). To account for the observed reversion of the young phialide-like element at permissive temperatures, the defect could be sought in the wall of the mutant (Vujicic and Muntanjola-Cvetkovic, 1973) or in the nuclear differentiation in the phialide of *A. niger* (Raper and Fennell, 1965). In *Penicillium claviforme,* the change from basipetal to acropetal cell proliferation was more clearly ascribed to the reversal of a cytoplasmic basophilic (RNA) gradient leading to an altered behaviour of daughter nuclei (Zachariah and Metitiri, 1971).

That the strength of polar differentiation of conidia is genetically-controlled is examplifed by conidia budding from the tips of long, aerial hyphae in wild type of *Neurospora crassa,* contrasting with the premature conidiation of short conidiophores in "crisp" mutants (Turian and Bianchi, 1972).

Microtubules are some way involved in conidiation of *Aspergillus nidulans* as shown by the morphogenetic consequences of selective tubulin mutations; conidiation-resistant mutants were able to conidiate in the presence of benomyl,

strongly suggesting that β_3-tubulin functions during spore formation (Morris *et al.*, 1984).

a^2 *Sporangia*

Structural changes accompany sporangial development in the "water moulds". During spore formation, the sporangium enters a brief phase when the cleavage furrows between spore initials become obscured, and under the light microscope the whole protoplasm takes on a uniform slightly granular appearance. This phase is termed the homogeneous phase (H-phase, Busgen, 1882, see Money and Brownlee, 1987). At the H-phase, spore delimitation is completed as the network of cleavage furrows in the sporangial cytoplasm fuses with the plasmalemma to produce continuous membrane-bound channels between the central vacuole and periplasmic space (Gay and Greenwood, 1966).

Coincident with the H-phase, in species of *Achlya* and *Saprolegnia,* the sporangial volume decreases by up to 13% and the previously concave sporangium-delimiting septum flexes into sporangium (Gay and Greenwood, 1966). These phenomena suggest that the sporangial pressure potential decreases during the H-phase as the tonoplast and plasmalemma are broken. Using an extracellular vibrating probe, Armbruster and Weisenseel (1983) measured an inward positive current at the tip of pre-H-phase sporangia of *Achlya debaryana,* which was replaced by a biphasic outward current of 2.5 $\mu A\ cm^{-2}$ during the H-phase. The inward current was subsequently restored. A similar collection of physiological changes accompanies the completion of cleavage in other aquatic fungi. Current patterns similar to those measured by Armbruster and Weisenseel (1983), including current efflux during cleavage, have been measured around thalli of *Blastocladiella emersonii* (Stump *et al.,* 1980). New results obtained with *A. debaryana* (Money and Brownlee, 1987) suggest that at least part of this current is carried by K^+ efflux. The significance of this inversed current patterns for differentiation from hyphae is still controversial (Harold *et al.,* 1987). A developmentally regulated enzyme, neutral protease, loses its affinity for Ca^{2+} ions at the transition vegetative — zoosporogenic hyphae in *Allomyces arbuscula* (Ojha and Turian, 1985).

a^3 *Basidiospores*

Basidiospores formation in higher Fungi involves cytoplasmic and wall layer synthesis and their interaction. Sterigmata appear to result from renewed tip growth at restricted loci in the basidial apex (McLaughlin, 1973). Basidiospore initiation is signalled by asymmetric sterigma tip expansion and it elongates by a mechanism homologous with hyphal tip polar growth (McLaughlin, 1977; Oláh and Reisinger, 1981). Sterigma outgrown from the apical dome of the basidium contain a dense cytoplasm with a cluster of vesicles near their tip. These must contribute to the polar

elongation of the usually four sterigma which reach their fullest length before spore initiation. Their wall is continuous with that of the basidium, but is thinner (bi- instead of four-layered).

b) *Algal exosporulation*

The polarized organization of vegetative filaments of the yellow green fresh water alga *Vaucheria* is lost at spore induction. The first indication that a vegetative filament is about to differentiate into a zoosporangium is a gradual darkening of its tip by migrating cytoplasmic organelles as they displace the large central vacuole. Many nuclei are involved in that polarized process; they accumulate through mass movement into the developing sporangia after release from the active microtubular band of the zone of cyclosis. Chloroplasts also flow into this region when the tip of the filament begins to enlarge. As in Fungi (a), there parallely occurs a dissipation of the gradient of vesicles in the enlarging tips of vegetative filaments arrested in their elongation. Soon after migration and accumulation of nuclei and chloroplasts into the filament tip, the pairs of centrioles associated with each nucleus begin to form flagella within internal flagellar vesicles. The flagella and their nuclei converge into many internal flagellar pools which then polarly migrate to the surface of the zoospore and become part of the plasma membrane. These events in the filament apex take place during the septation of the vegetative filament. At the time of zoospore release, all nuclei are positioned just beneath the zoospore surface and are intimately associated with pairs of external flagella (Ott and Brown, 1974b).

Positional control of algal differentiation has been reviewed in 1984 by Waaland who referred to Kataoka's work (1975) for tip organization in *Vaucheria* in which branching was induced by unilateral illumination.

2. *Bipolar patterns*

a) *Mating types*

The bipolar sexualization in the *Allomyces* generative of sexually compatible gametes corresponds to a homothallic condition. By contrast, in dimictic or heterothallic forms of Fungi, a haploid individual can never breed with more than 50% of the whole population but can always mate with half the progeny of the same zygote (Burnett, 1975). Such mating systems thus favour the opportunity for outcrossing in preventing the selfing of a single haploid. They are functioning through a unifactorial, bipolar expression of one of the two alleles A/a at the same locus as best illustrated by the classical, heterothallic *Neurospora crassa* and *N. sitophila* (Whitehouse, 1949).

Those phenomena described as bipolar mating systems correspond to homogenic incompatibility defined by Esser and Blaich (1973) as the inhibition of zygote formation between partners of the same species due to the heterogeneity (pluripolarity)

of their incompatibility loci. Such a situation has been analyzed in different strains of *Podospora anserina* and appears to extend to other fungal species.

Relatively little is still known about the chemical basis of the bipolar compatibility versus incompatibility of heterothallic Ascomycetes. A complementary mechanism involving A and a mating gene products acting as the "key-lock system" has been envisaged for zygote formation (Esser and Kuenen, 1967); this assumption implies that in the incompatibility combination, both partners form gene-like products unable to complement.

Bipolar compatibilities or incompatibilities are independently controlled of the sexual phenotypic traits, as examplified by the heterothallic *Neurospora* spp. where a female coiled structure, the ascogonium, can statistically be either of the (+) or (−) mating type and thus conjugates with (−) or (+) conidia respectively (Turian, 1978*b*). This bipolar compatibility or mating system is also effective in Zygomycetes (Mucorales +/−), the yeasts (α/a) and many higher Fungi (A/a). However, an exceptional situation, the *tetra*polarity, can be preeminent in certain species of these higher Fungi, as examplified by the genetically well-known "split gills" *Schizophyllum commune.* Such tetrapolarity is controlled by two allelic pairs designated as Aa and Bb. Full compatibility and fertility (fruiting bodies) is only assumed by the fully complementary formula AaBb (Raper, 1966).

The simpler bipolar system of yeasts controlling transcription-translation of mating type substances is not fully fixed in homothallic strains. It is controlled by the changing expression of mating type substances known as polypeptides (Herskowitz, 1987). In their vegetative phase, yeasts are predominantly diploid cells heterozygous for the mating-type locus MAT. In heterothallic strains, germination and cell division result in clones of stable haploids of either *a* or α mating type. However, in homothallic strains a single a or α spore will rapidly give rise to *a*/α diploid without having to encounter cells of different clonal origin and mating type. When germinated in isolation from other cells, homothallic spores with α mating type, for example, divide by budding to produce a mother cell and a daughter cell, both of which retain the original α mating type. During their subsequent cell divisions, the mother cell switches mating type and produces a pair of a cells, whereas the daughter retains its mating type and produces a pair of α cells. Cells of opposite mating type now arrest each other in G_1 by secreting one pheromone (*a* or α) and by responding to the other (see Saier, and Jacobson, 1984). During the pheromone-induced G_1 arrest, the cells elongate, becoming pear-shaped ("schmooing", Fig. 18 B) and eventually fuse to produce a pair of diploids now heterozygous while recovering an ovoid cellular shape to further divide by budding during the diplophase (Cross *et al.,* 1988).

It remains the question of how does a cell divide to produce daughter cells which have different developmental fates? According to Nasmyth and Shore (1987), the sequence of developmental events are regulated at the level of transcription factors

encoded by *Switching* (*SWI*) genes which activate an endonuclease gene (*HO*) at a precise stage in the cell cycle of mother cells. Since *HO* is only activated in one of the sister cells after division (the mother), adjacent cells of opposite mating type are generated which respond to each others' secreted pheromones by inducing genes involved in conjugation and this leads to the formation of a diploid.

b) *Sexual disjunction*

Rigid rules govern the placement of reproductive organs in lower plants. In mosses, with the exception of *Sphagnum,* the antheridia and archegonia are formed at the tip of the stem but not by the apical cell itself. Interestingly, in conifers the position of male and female cones is also subject to relatively strict rules: at the onset of its sexual maturity, *Pinus sylvestris* initially produces some female cones on leading shoots; male cones are then produced basitonically on lower branches, female cones being produced acrotonically (Carr, 1984). These positional rules in conifers are maintained even when cones are induced experimentally by treatment by gibberellins (Pharis, 1978).

In higher plants, phyllotaxis increases in complexity with the onset of flowering (Carr, 1984). The same apex can produce first a suite of leaves, then a closely-set suite of flowers and subsequently reverse to leave production. Informational controls regulate this reciprocal positioning of floral parts and leaf primordia. There are also examples of anisophylly and anisoclady (see in Carr, 1984). Positioning of stamens (androceum) relative to that of the pistil (gynaeceum) is described in classical botany as either epigynous or hypogynous.

A similar positional pattern is shown by the gametangia of the aquatic molds of the genus *Allomyces.* In that most experimentally studied system, the bipolar phenotypic disjunction of two sexually distinct multinucleate cytoplasmic "territories" (coenocytes) in the apices of the gametophytic haploid hyphae is reminiscent of the unequal division described by Bünning (1957) as a basic mechanism of cell differentiation. The fact that the bipolar axis is specifically (genetically) oriented, as shown by the epigynous *versus* hypogynous arrangement of the gametangial couples in *A. macrogynus* versus *A. arbuscula* (Emerson, 1955) adds to the interest not to speak of the complexity of the case (see Plate, p. 3).

As first step in this double differentiation, dissipation of the monopolar organization of the vegetative hyphal apices precedes the specifically oriented, gradiential reorganization in the enlarging, now club-like hyphal apices (Turian, 1969). The direction of such a chemo-structural gradient should therefore be under genetical control, namely that of some bit of DNA considered as the positional information. The transcription of this information is initially triggered — and the positioning gene(s) switched-on — by environmental stimuli such as starvation in aerated liquid media.

Out of its apparent cytoplasmic uniformity at inception, double differentiation proceeds by the inversely oriented sorting out of the characteristic, functional structures of male and female cells. This DNA-controlled positioning process (Fig. 24 C; Ojha and Turian, 1971) can tentatively be grasped from both extremities of its span: (1) at the start, with the DNA and its positioning effect on cytoplasmic effectors, leading to a polarized expression of the sexual information; (2) at termination, with the characteristic differential features of the genetic expression, the four most prominent features known which contribute positively or negatively to the sorting out of male from female gametangia, being (Turian, 1975): (1) the nuclei (also nucleoli), smaller and more numerous in the male gametangia; (2) the ribosomal nuclear caps, more massive and with more RNA in the female gametangia; (3) the mitochondria, apparently less numerous and often poorly cristated in the male gametangia; and (4) the orange pigment (γ-carotene) only in the lipid granules of the male gametangia. The larger size of the female gametangia reflects their more efficient synthetic power.

It has been suggested that it is an AT-rich cytoplasmic DNA which could function as positioning DNA by orienting a basophilic, RNA-proteinic gradient cytochemically detected along the axis of the differentiating gametangia (gradient head in the female "territory") as most visibly expressed at their nuclear cap forming stage (Fig. 24 A; Turian, 1969). Such massive concentration of ribosomal RNA in the female gametangia and the free gametes was confirmed by their twice higher RNA/DNA ratios compared to male cells. To meet the need for more energy to produce a RNA and protein richer female cell should request efficient mitochondria. Those were shown to be more internally developed (cristae) in female cells (Turian, 1969). Additional evidence for the oxidative deficiency of "male mitochondria" came from cytochrome oxidase measurements and the masculinizing effects of acridine compounds (antimitochondrial agents) by suppression of the female gametangial differentiation (Turian, 1975; Olson, 1984).

A dynamic scheme involving gene-controlled polar liberation in the hyphal cytoplasm of episome-like factors activating mitochondrial competence in presumptive female zone has been proposed to integrate all available genotypic and phenoptypic data concerning this polarized sexual disjunction (Fig. 24 B; Turian, 1969, 1975). Superposed on the increased respiratory competence developed by the "feminizing mitochondria" in response to oxygen tension, there occurs a gene-induced oxidative deficiency of the mitochondria present in the presumptive male "territory", through the acridine-like action of a polarly distributed suppressive factor. Triggered activation of the positional DNA would be necessary to insure such polarized spreading of the suppressive masculinizing factor. The "mas" gene of a male mutant of *A. arbuscula* could well be the gene producing the suppressive factor and the «pol» gene the true positioning DNA to achieve epi-*versus* hypogyny. The control of a polar

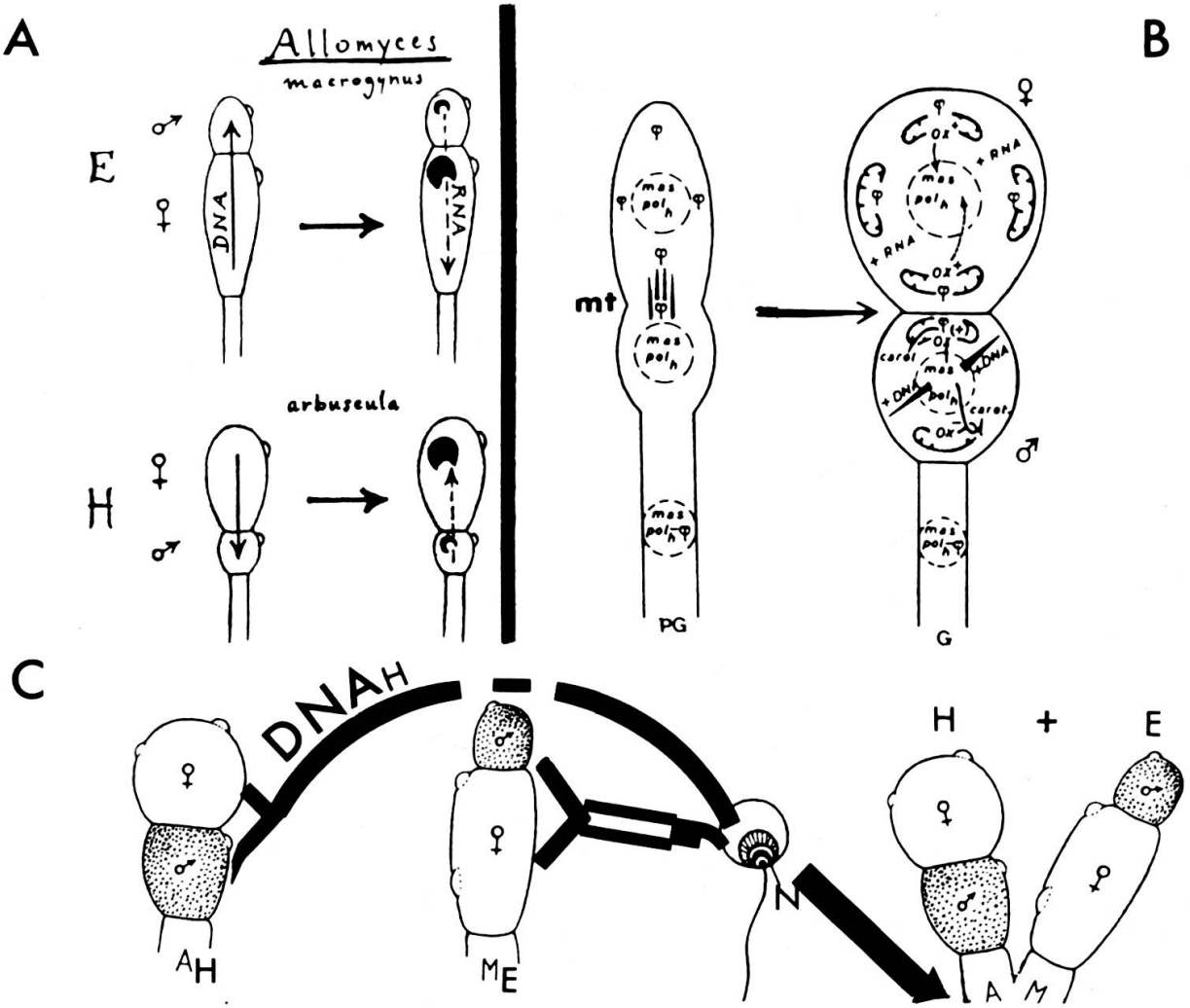

FIG. 24.

Polar positioning of *Allomyces* gametangia.

(A) Specifically inverse bipolar gradiential segregation of DNA and RNA at gametangial differentiation of the epi (E) *versus* hypogynous (H) species of *Allomyces*. "Male-head" gradients of DNA content (Feulgen-Giemsa stainings) — mitogenic capability are first produced (left), followed by the inverse "female-head" gradients of RNA content (toluidine blue staining) expressed in their larger nuclear caps ("ribosomal-crescent bags") at the pregametic stages. From Turian, The Nucleus, 1961.

(B) Model of the biochemical events occurring at the bipolar segregation of male and female gametangial cytoplasms founded on (1) the masculinizing effect of acridines or of presumed similarly natural compound(s) acting as suppressors of feminizing (φ-) prooxidative factors favoring an increase of the RNA/DNA ratios in the female gametangia (see A); (2) the polar positioning of the suppressive factor(s) along tracks of microtubules (mt)-microfilaments between the progametangia (PG); the antitubular - antifilament compounds dissipate such polar segregation, and, spreading the female suppressor(s), masculinize the gametangial (G) zone. From Turian, 1969 and Turian and Ojha, 1987.

(C) Transfer of positional DNA from the normally 100% hypogynous (H) species *A. arbuscula* to the normally 100% epigynous (E) *A. macrogynus* grown and sexually differentiated from the wall-less meiospores (N = nucleus) germinated in the presence of the heterologous DNA_H. The ratios of hypogenous *versus* epigynous gametangial arrangements differentiated from the same main hypha (see right of the Fig. C) was the highest in the DNA transfer direction from $_H$ to $_E$. Reciprocal DNA-positioning effects — from DNA_E — were less efficient. The highest ratios obtained corresponded to those shown by both the natural hybrid *A. javanicus* ($_H \times _E$) and certain strains issued from *in vitro* hybridizations (Emerson, 1955) spanning from nearly fully masculinized to nearly fully feminized strains through bisexual hybrids bearing inversely positioned gametangial couples. Dotted gametangia are γ-carotene - pigmented males (*carot.* in B). Adapted from Ojha and Turian, 1971.

disjunction of gametangial territories could thus be effected through the gradiential segregation of a respiration inhibitory factor.

The chemo-mechanical mediators of genetical polarity in the *Allomyces* being still unkown and, in view of the recent biochemical trend to attribute such a role to the cytoskeleton (Quatrano, 1978; Fulton, 1984), it became tempting to ascribe the vectorial cytoplasmic movement to microfibrillar-microtubular elements of the cytoskeleton. Recently, it has been found that the genetically-controlled inverse bipolar sexualizing gradient of the *Allomyces* can be dissipated by adequate concentrations of the antiactin cytochalasin E or the antitubulins DMSO and benlate (Turian and Ojha, 1987). These effects suggest that the presumed disruption of bipolarizing tracks allows the dominant male tendency (suppressive effect) to overflow the presumptive female "territories" in the sexually differentiating hyphal apices of *both* epi- and hypogynous species. A first conclusion was that "DNA-controlled inverse bipolarity is mediated by the inversely-directed function of filamentous actin and/or tubulin microstructures vectorially conveying the sex-determining elicitors".

C. APICO-BASAL DIFFERENTIATIONS — HETEROBIPOLAR AXIATION

There are many instances in plants (see Miller and Bassel, 1980) in which cell differentiation is initiated by the establishment of a polar axis preceding the unequal cell division occurring transverse to that axis (Fig. 26).

Cell differences arise as result of influences, possibly electric potential gradients which bring down unequal distribution of cytoplasmic factors in the mother cell before that division occurs. Electrical potential differences, resulting in the electrophoresis of information molecules, are thus important in bipolarity and unequal division in eggs and others cells (Smith and Grierson, 1982).

Issued from a geometrically asymmetric cell division, the daughter cells are of different sizes and develop directly into different cell types. Bünning (1957) described several examples of this phenomenon and advanced what is probably the most widely-held view of the morphogenetic function of asymmetric cell division. This hypothesis states that there is polar cytoplasmic differentiation within the mother cell. When the nucleus migrates to one end of the cell, and an asymmetric cell division occurs, the two resulting nuclei reside in different cytoplasms. Since the cytoplasmic environment regulates which genes are expressed and which are repressed at a given time, the different cytoplasmic environments of the two nuclei call forth the expression of different genetic informations from each and result in cellular differentiation.

Some important questions exist concerning the detailed operation of this hypothetical scheme for the induction of cellular differentiation. Two major points are: what is the origin of polarity in the mother cell; and why is geometrical asymmetry of cytokinesis an obligate step in so many instances? Polarity may exist in the mother

cell as a preexisting, persistant state, or it may arise epigenetically in a mother cell which initially is non-polar. An epigenetic origin of polarity appears to be the situation, for example, in zygotes of the Fucales. The egg is apolar, whereas a polar axis may be established in the zygote after fertilization by the imposition of any of several external gradients — for example, in pH, ionic concentration, electrical field or light intensity (Jaffe 1968, 1970; Quatrano, 1978). This axis determines a polarized redistribution of cellular organelles, and the mitotic spindle is oriented so that cell division occurs transverse to the polar axis (Fig. 20 A). The cell at one pole of the axis develops into a multicellular rhizoid, whereas the other cell and its derivatives become the thallus. In contrast to this situation, one may conceive of a cell which has a pre-existing polar axis — perhaps having a structural basis — which is persistant and relatively unaffected by external conditions.

The molecules or particles accumulated in one cell could be of two types: (a) cell-specific components which immediately distinguish the new cell from its parents, or (b) components of a more "determinative" nature which would commit the new cell and possibly its progeny to a particular developmental pathway, ultimately leading to the expression of a specific cell or tissue type.

1. *Caulobacterial cells (flagellate-stalk poles)*

A unique feature of these so-called prosthecate bacteria is that they carry out obligatory well-defined differentiation changes during their dimorphic cell cycle. A new cycle is initiated with a new round of chromosome duplication by the first type of so-called stalked cell; their stalk or prostheca is a polar extension of the lipopolysaccharide and mucopeptide layer of the cell wall which provides attachment of the cell for some suitable substrate. After the main body of the cell has enlarged to form the predivisional cell an asymmetric division follows to give one daughter cell that retains the stalk and another which develops a flagellum and fimbriae becoming a swarmer cell.

Under normal growth conditions, a new stalked cell synthesizes an intracytoplasmic structure at the pole opposite the stalk, and this process represents the first step in a cycle of polar differentiation. It has been suggested that this structure is a membranous organelle that can be distinguished from cell mesosomes by the lack of continuity with the cytoplasmic membrane. The same type of membranous organelle is found at the stalked pole of the mature cell. We are still unsure of the nature of this organelle, because electron microscopic analysis does not consistently reveal a discrete, membranous structure in the region of the polar organelle.

The second occurrence in the differentiation process is the formation of a single flagellum and several pili also at the pole opposite the existing stalk, thus generating a typically bipolar pattern. Binary fission yields a sessile stalked cell and a motile swarmer cell (Fig. 25 B). The daughter swarmer cell must differentiate into a stalked

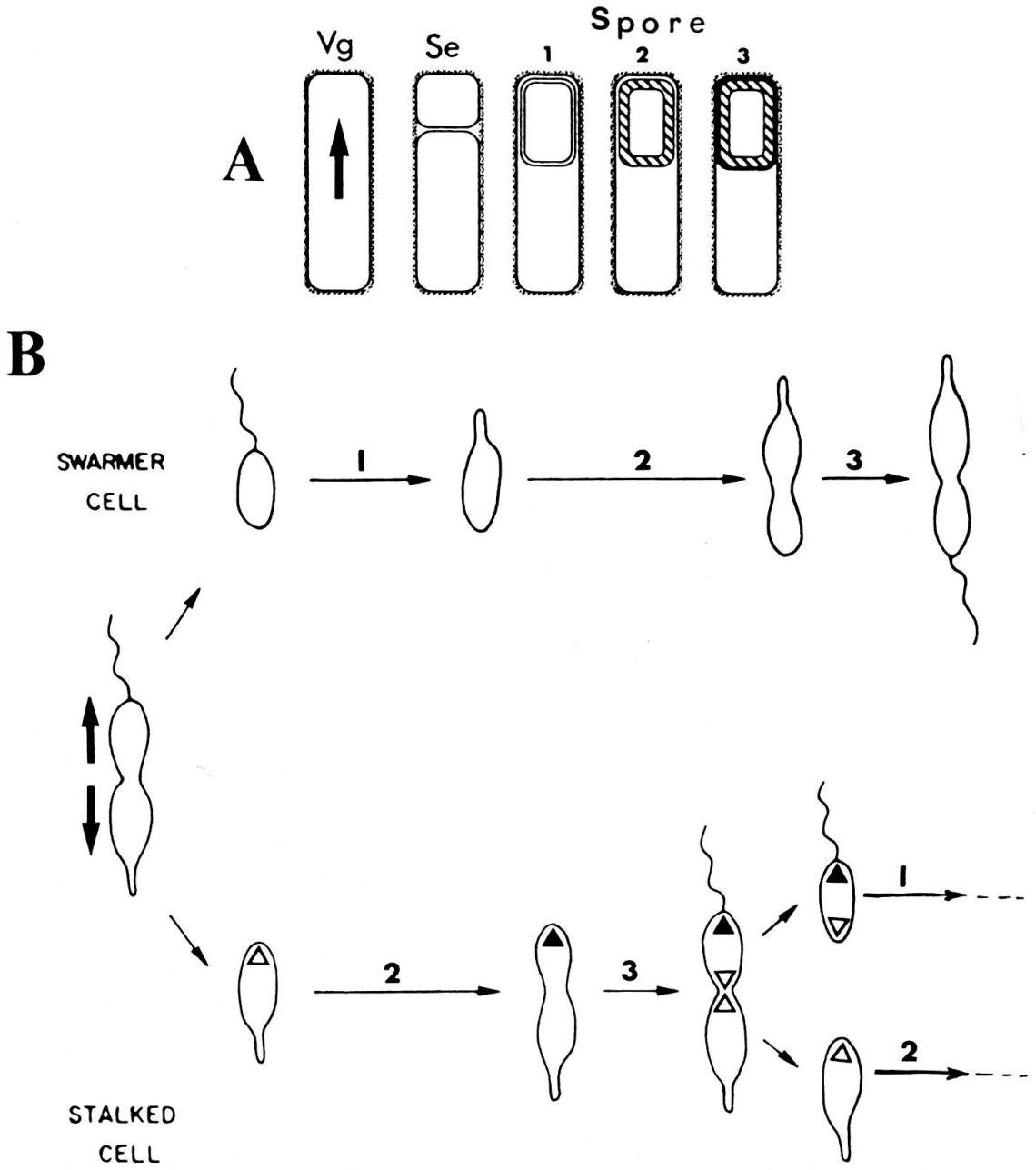

FIG. 25.

Polar differentiations in Bacteria.

(A) Monopolar differentiation at *Bacillus* sporulation: asymmetrical division (arrow) of the undifferentiated vegetative cell (Vg); septation (Se) followed by spore differentiation from the protoplast stage (1) through cortex (2) to coat formation and maturation (3). Adapted from J. Errington, Nature 333: 399 (1988).

(B) Bipolar differentiation in *Caulobacter crescentus:* asymmetric cell division (opposite arrows) producing the swarmer cell and the stalked cell. Repeated divisions of the stalked cell produce flagellated swarmer cells (2, 3); the former cells are then converted (1) to stalked cells. Hypothetical organizational centres (open triangles), laid down in division, become sites for polar assembly (closed triangles) in the next cell cycle. Adapted from Newton *et al.,* 1985.

cell before it is capable of cell division. This transition, the third step in the differentiation process, involves the deposition of new cell wall material at the point of attachment of the flagellum to the cell. The preexisting polar membrane structure now constitutes the interior of the newly forming stalk, where the flagellum can be found as an appendage at the tip of the stalk (Shapiro *et al.*, 1971).

The two cell types of the dimorphic cell cycle are out of phase in their cell cycles: the stalked cells commence a new cycle immediately with the initiation of a new round of chromosome replication; the swarmer cells, however, are immature and of necessity are required to undergo some as yet unknown differentiating steps before becoming stalked mother cells and embarking on division. Most obvious is the repression of DNA replication in these cells, with the event proceeding only when stalk development has taken place some 30 minutes after cell division.

Several features of developmental regulation in *C. crescentus* distinguish it from other systems. First, a "terminally" differentiated cell type is not formed; the swarmer cell is not some kind of a motile spore, since it is metabolically active and grows in preparation for the initiation of DNA synthesis. Second, culture conditions, such as nutrient deprivation, are not required to trigger any of the developmental stages; and third, cell-cell interactions have not been observed to play a role in development. The dimorphic cell cycle in *C. crescentus* cell represents a "stripped down" version of cell differentiation in which the temporal and spatial events are cell autonomous and occur repeatedly as part of a vegetative cell cycle (Newton *et al.*, 1985).

The two progeny cells are differentiated with respect to cell structure, but also they are programmed to follow different cell cycles; the stalked cell initiates DNA synthesis immediately after division, whereas the swarmer cell enters a presynthetic gap (G1) required for differentiation into a stalked cell. The pattern of protein synthesis in the two cells is also different and organization of the predivisional cell involves signals for positioning of proteins to one portion of the cell opposed to another (Shapiro, 1985).

A functional differentiation of polar membrane domains has been described by Newton *et al.* (1985): the assembly of the surface structures on the incipient swarmer cell always occurs at the new cell pole, and the mutational analysis outlined above showed that formation of the assembly sites requires completion of a late step(s) in cell division; the position of these sites is specified by "organizational centers" that are laid down as part of the division site in the cell cycle before assembly is initiated. As a consequence of these events, the new cell pole should be stably differentiated from the remainder of the cell envelope.

An important problem in localized assembly is to identify the stage of translocation at which the subunits of the surface structures are targeted to the polar membrane domain. Pulse-chase experiments have shown that newly synthesized flagellin in *C. crescentus* cells is translocated for assembly in the sequence soluble pool — mem-

brane pool — assembled filament. In very short pulse-labelling experiments, followed by radioimmunoassay for labelled flagellin in polar and non polar vesicles, almost the entire membrane pool flagellin was found in the polar vesicles. Thus, flagellin is apparently targeted to the cell pole as a relatively early stage of translocation to the membrane (Newton *et al.*, 1985).

Recently, monoclonal antibodies have been isolated (Sommer *et al.*, 1986) that bind either uniformly to the cell surface or to one of the cell poles. The antibodies specific for proteins at the flagellated cell pole and at the stalked cell pole verify the presence of stable membrane domains in the intact cell. These antibodies are being used as probe to study the mechanism of polar membrane protein translocation.

Mutants that are blocked at various stages of morphogenesis offer a promising approach toward understanding the relation between gene function and the concerted series of events that eventually results in bipolar differentiation.

2. *Fungal cells (rhizoid-hyphal poles)*

A flagellated spore of an aquatic Phycomycete such as *Allomyces* spp., whether a zoospore, meiospore or zygote, withdraws its flagellum(a), rounds up, and encysts (Olson, 1984). At cyst germination, a germ tube grows out from one pole of the cyst and rapidly branches to establish the rhizoidal system. Following the establishment of that rhizoidal system, a second much broader germ tube — in which flows an inward positive electric current (Youatt *et al.*, 1988) — grows out of the cyst at the pole opposite (180°) the point of origin of the rhizoidal system (Fig. 26 B). This second germ tube forms the basal cell of the thallus which then branches distally into dichotomous hyphae (Turian, 1985*b*). In *Allomyces* germlings, the hyphal pole can be deviated from its opposite positioning (normal bipolarity) by mechanical (centrifugation) or chemical (cycloheximide) treatments (Turian, 1958, 1962). This could result from a disruption of the cytoskeleton (protein?)-controlled bipolar axiation.

3. *Algal cells*

a) *Eggs (rhizoid-thallic poles)*

A basic part of algal pattern formation is the development of polarity which is itself closely connected to the formation of electrical and chemical gradients. The Fucales have provided classical materials for studies of the embryogeny of seaweeds and more is known about the ova eggs and young embryos of these plants than of any other group. They have afforded botanists materials which are comparable in many respects with the eggs of various amphibians (VII.C.6a[5]).

The fertilized egg of *Fucus* is a spherical and relatively undifferentiated body. It contains homogeneously distributed brownish green photosynthetic plastids and is naked before fertilization; but immediately after there is a secretion of mucilage

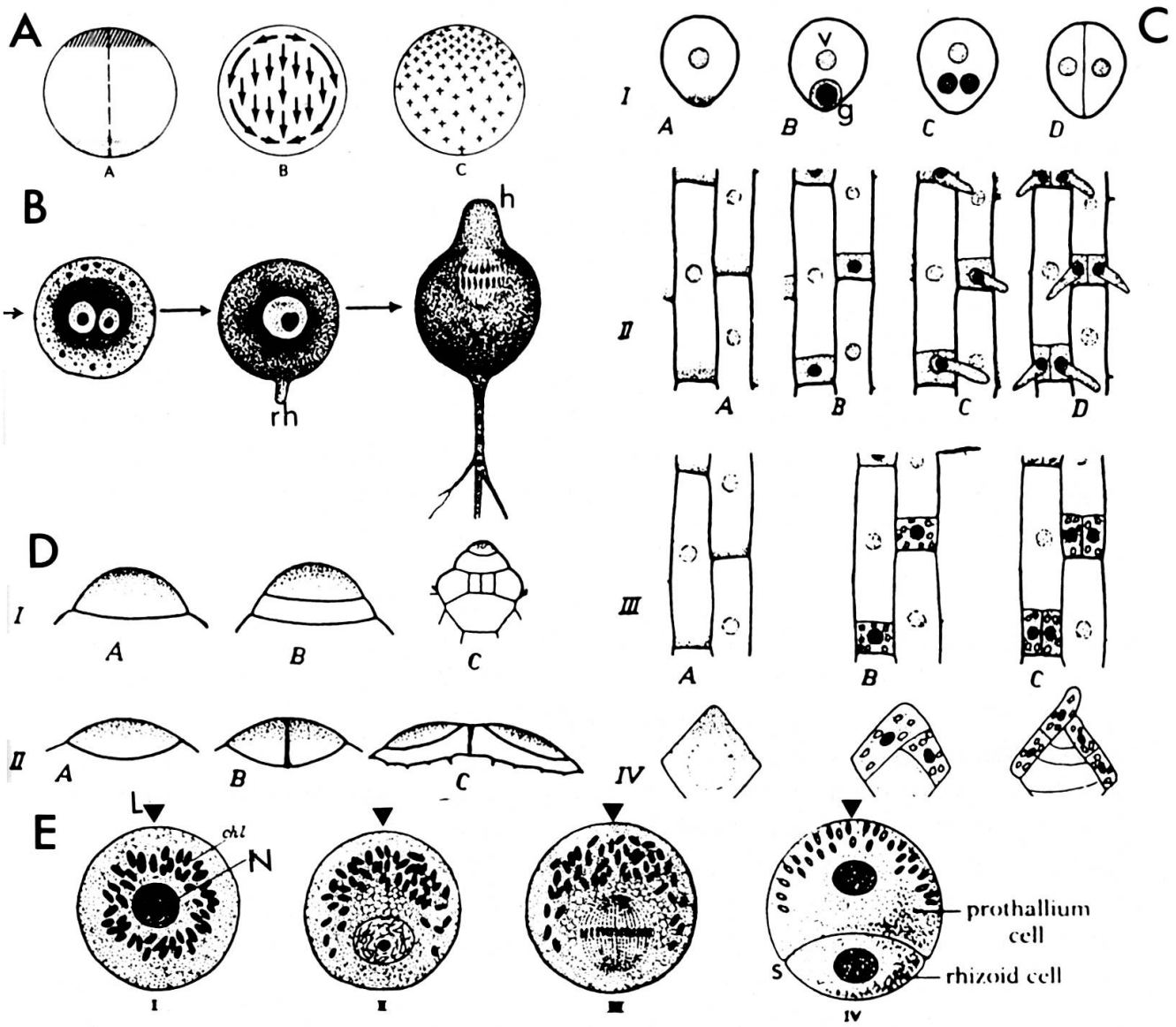

FIG. 26.

Cell asymmetry generative of bipolar differentiations.

(A) Types of cell polarization: (A) polar field polarity; (B) structural orientation polarity; (C) gradient polarity. From Grant, 1978.

(B) Gradient polarity with internal reorientation of organelles (mitochondria, nuclear positioning, etc.) during the three last stages of zygote germination in the *Allomyces:* uniform (isometric) state monopolar rhizoid (rh) emergence bipolar stage with hyphal (h) outgrowth. After Turian, 1969.

(C) Unequal and polar cell divisions: (I) in pollen grain (A) gradient of dots (ribosomes?) Grant's C type; (B) unequal division producing a vegetative cell (v) and a generative cell (g); (C) further division of generative nucleus; (D) unusual septation in the direction of the polar gradient producing no differentiation. (II) Differentiation of the monocotyledonous type of root hairs formation: (A) gradient of protoplasmic density; (B) trichoblasts generative of unequal division; (C) developing root hairs; (D) division of trichoblasts in the direction of polarity gradient. (III) Monocotyledonous type of stomata formation, similar to that of root hairs. (IV) Unequal divisions in the leaf cells of *Sphagnum* with dots again showing a protoplasmic gradient. After Bünning, 1952.

(D) Polarity as basis of differentiation examplified by: (I) stages (A-C) of division of an apical *Chara* cell producing two cells with different contents (B). (II) Stages (A-C) of division of an apical *Dictyota* cell in the direction of the axis of polarity, thereby producing two equal, undifferentiated cells. After Bünning, 1952.

(E) Light (L)-induced structural orientation polarity in the germinating spore of *Equisetum:* (I) unpolarized spore with central nucleus (N) and uniformly distributed chloroplasts (chl); (II) early polarization by changed positions of plastids and nucleus; (III) first nuclear division followed by (IV) asymmetric septation(s) preceding rhizoid outgrowth from the colorless basal compartment. After Nienburg, 1924, adapted from Sinnott, 1960 (I-III) and Grant, 1978 (IV).

which hardens to form a close-fitting, cellulose-like wall (Wardlaw, 1952). This quantitative change — still growth — is followed by the first qualitative change — differentiation — which is the appearance at one peripheral point of a rhizoidal outgrowth. The first cell division then takes place in the plane at right angles to the emerging rhizoid (Fig. 20 A). This highly asymmetric division is preceded by development of polarity in the egg, with the consequence that each daughter nucleus is delivered from the division into a different cytoplasmic environment; this in itself will tend to fix and accentuate the polarity. The division of the single egg cell will therefore give rise to daughter cells with different potentialities, one delimiting the rhizoid, the other developing into the frond or green thallus. The rhizoidal area is characterized by the intracellular accumulation of mitochondria, Golgi and associated vesicles, as well as the localization of sulfated polysaccharides in the cell wall. Quatrano (1978) has used the sulfated polysaccharide fucoidin as a localized, cell specific product to study the events required for its incorporation into the cell wall at a site determined by an extracellular gradient.

The initial generation of polarity in an initially *non*polar cell such as the *Fucus* egg is provoked by many types of external factors (pH, light, etc.). In the absence of any external stimulus the polarized outgrowth takes place at a random location on the surface of the cell, but with a substantial delay. According to Meinhardt's model (1984), "there is a competition between each surface element of the cell and one area will eventually win. The smaller the externally-imposed asymmetry is, the lower it takes for one part of the cell to dominate".

On the assumption that it was not already present in the unfertilized egg, polarity of the developing germ tube is determined by the factors which determine the position of the rhizoid. Colchicine does not block axis fixation or photopolarization, but prevents the first oriented cell division (perpendicular to the rhizoid axis). Therefore, some cytoskeletal basis other than microtubules and the spindle apparatus is responsible for the original polar axis. It follows that the plane of the first cell division is predetermined by the previously set rhizoid axis. This polar axis fixation and the localized accumulation of macromolecules can be experimentally directed by light. In an attempt to separate these events in time from each other, zygotes exposed to unilateral light were treated with reversible inhibitors of rhizoid formation. When the source of orienting light was rotated after untreated controls had established a fixed axis, rhizoids formed from the shaded side of the first orienting light, indicating that a fixed axis was established in the presence of the rhizoid inhibitor. Cycloheximide and sucrose-treated zygotes responded in this manner i.e. these inhibitors uncoupled fixation from localization. Oppositely, cultures treated with cytochalasin B (CB) responded to the second orienting light indicating that this inhibitor prevented axis fixation. However, also CB prevents axis establishment and axis fixation by light, it does not disrupt the orientation of a previously induced axis (Quatrano, 1973). Moreover, it was experimentally shown (Quatrano, 1978) that the prevention of axis

fixation by CB results in the lack of fucoidin localization due to an apparent disruption of the directed transport of Golgi and associated vesicles.

What other localized events occur at the presumptive site of rhizoid formation before and during axis fixation, and what is the event blocked by CB? The endogenous electric currents associated with cell polarization are disrupted suggesting that a pattern of actin microfilaments may transport Golgi-derived vesicles containing calcium channels selectively to the growing tip (Brawley and Robinson, 1985).

Nuccitelli (1978) has shown that the influx of ions and secretion of wall material occur at the presumptive rhizoid site *before* fixation of a light-induced polar axis. *Pelvetia fastigiata* zygotes exhibit both an accumulation of cytoplasmic vesicles and a clear area between the plasma membrane and cell wall at the rhizoid pole several hours prior to rhizoid emergence. This "cortical clearing" is most likely material for cell wall formation accumulating intracellularly and being secreted. In addition to cortical clearing, local fluxes of inward directing ions clearly precede rhizoid formation in *Pelvetia,* and can be localized in the plasma membrane regions of the presumptive rhizoid site before axis fixation. An ultrasensitive vibrating probe (Jaffe and Nuccitelli, 1977) was utilized to detect currents entering and leaving various surface regions. Using the probe, Nuccitelli (1978) demonstrated that shortly after fertilization the spatial current pattern around the *Pelvetia* zygote is shifted between several inward current regions. However, as the time of fixation approaches, these inward currents were concentrated at the presumptive site of rhizoid formation.

As summarized by Quatrano (1978), the time-course of events within the algal zygote could be the following: 1) The detection of inward direction current in the plasma membrane following fertilization. 2) Inward currents directed toward the presumptive site of rhizoid formation by an external gradient e.g. light; this represents the first sign of localization. 3) Accumulation and secretion of vesicles containing cell wall material (cortical clearing). 4) A process sensitive to cytochalasin B and insensitive to cytochalasin H and sucrose which fixes this site of intracellular ion accumulation and vesicles secretion for subsequent localization of particles and organelles needed for wall extension. 5) The incorporation of the acidic polysaccharide fucoidin and other polysaccharidic materials into the rhizoid wall.

b) *Stalks (rhizoid-cap poles)*

The inherent polarity of cells of algal threads of the fresh-water *Cladophora glomerata* was demonstrated by regenerative behaviour following temporary plasmolysis in 20% salt solution (Czaja, 1930). Deplasmolyzed cells began to enlarge by breaking out of their wall, and proceeded to regenerate new filaments; then, at the basal end of each cell, a new rhizoid was formed and from the apical end, a new thallus. Reversal of polarity in the threads of *Cladophora* was produced by centrifugation, showing again the close relation between the distribution of materials

in the cytoplasm and the polarity of cells. Similarly, in bits of tissue cut from *Enteromorpha,* the cells near the apical portion of the piece regenerated papilla-like structures while the cells at the base formed rhizoids (Müller-Stoll, 1952).

A case where the cell obviously has a bipolar character is the marine green alga *Acetabularia* with its cap at one end and its rhizoid at the other (Fig. 22). A fragment derived from the center of the cell will regenerate both basal and apical ends (Hämmerling, 1955). Much speculation has resulted from experiments with *Acetabularia mediterranea* and *A. Wettsteinii.* The thallus of this plant, described as uninucleate in the vegetative state, shows considerable differentiation, consisting of a basal rhizoidal portion with the nucleus, a stalk with sterile whorls or hairs, and an umbrella-shaped apical part. That the nuclear products are specific is concluded from formative effects obtained by grafting a piece of stalk of *Acetabularia mediterranea* on a rhizoid containing the nucleus of *Acetabularia wettsteinii.* Development of the scion then assumed the character of the stalk. It was then concluded that the polarity and formative effects were not due to an intimate structure of the cytoplasm nor to a metabolic gradient. The mechanism which controls the flow of the substances and their formative effects in the cytoplasm remains unexplained.

Cap differentiation in *Acetabularia* depends upon the release of "morphogenetic substances" (believed to be RNA) from the nucleus but it does not require the presence of this organelle for expression. Anucleated stalks can form the species-specific cap as well as regulate the levels of certain enzymes, suggesting that long-lived or stable mRNA is implicated in the genetic control of this localized differentiation (Quatrano, 1978).

4. *Cryptogamic spores (rhizoid-thallic poles)*

Events at cryptogamic spore germination illustrate well Bünning's hypothesis (1957) stating that there is polar cytoplasmic differentiation within the mother cell. A few important questions still remain about the operation of this hypothetical scheme for the induction of cellular differentiation: what is the origin of polarity in the mother cell? Why is geometrical asymmetry of cytokinesis an obligate step in so many instances?

From a swelling moss spore can emerge a primary rhizoid and/or a chloronema (Heitz, 1940). The young outgrown chloronema pushes out on the side of the spore toward the light, and the rhizoid forms at the opposite end, indicating that here, as in the *Fucus* egg, its polarity is determined by light. In several moss species, Fitting (1949) was able to reverse this polarity by reversing the direction of the light. In this way the young protonema becomes converted into a rhizoid. The tip of moss protonema responds phototropically and polarotropically, i.e. it perceives the vibration plane of the light. The polarotropic orientation of primary protonemal

growth of *Physcomitrella* depends on the wave length and the photon flux rate of monochromatic light, and the cell elongates parallely to the electrical vector of plane-polarized light in blue light (Schnepf, 1986).

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

The moss protonema has been considered as an hormonally-controlled "morphogenetic system" in which an electric current was measured entering the tip of each growing region, namely the main filaments and side branches (Bopp, 1980). In an electric field the spores of *Funaria* tend to form the primary rhizoids toward the positive electrode. This response appears to be mediated by active calcium ion uptake as suggested by the predominant formation of those elongating structures towards the side of highest concentration in the Ca^{2+}-ionophore A 23187 (Chen and Jaffe, 1979). By contrast, subsequent growth of both rhizoids and chloronemata was directed towards the negative electrode. Other environmental orienting effects have been described by Schnepf (1982).

The spore of the horsetail *Equisetum* shows no predetermined, external or internal polarity and its germination is followed by division into two unequal cells. Light was shown to be the directing factor in the division of this apolar spore (Stahl, 1885). A redistribution of cytoplasmic material first takes place, especially an aggregation of chloroplasts on the illuminated side (Nienburg, 1924). Then only occurs the alignment of the nuclear spindle parallel to the direction of light (Fig. 26 E) followed by unequal division of the cell by a wall laid down at right angles to the gradient of light absorption. The axis of the division figure and the daughter nuclei become arranged in such a way that the more strongly illuminated daughter cell becomes the primary prothallial cell, and the one on the darker side, the rhizoid cell. The point of emergence is highly basophilic and numerous mitochondria appear in differentiating rhizoids (Nakazawa, 1956-60). The first, larger cell is chloroplast-rich and will later give rise to the plant shoot, the second smaller cell is chloroplast-poor and will give rise to the root-like rhizoids. The spores of *Equisetum* require only a few seconds of irradiation to induce localized germination several hours later (Haupt, 1957). The very short duration of light action and its low intensity necessary to give rise to a certain spatial development in the cell sometime later lead therefore to conclude that this morphogenetically effective physical factor functions only as signal and does not supply the cell with the necessary energy for the subsequent reactions (Weisenseel and Kicherer, 1981). Preceding stable polarization of intrasporal content, there has been observed a period of labile polarization when the orienting effect of illumination could still be negated by subsequent illumination from the opposite

direction (Mosebach, 1943). The spores of *Equisetum* respond to applied electric fields as the zygotes of *Fucus* and *Pelvetia* by germinating in a parallel direction to the field (Bentrup, 1968).

According to Miller and Bassel (1980), "polarity may exist in the fern mother cell as a *pre*existing, persistant state, or it may arise *epi*genetically in the mother cell which initially is non-polar". As a first experimental answer, these authors found that in most caffeine-treated spores, the nucleus moved back to the center of the spore in which cell wall formation and rhizoid differentiation were both blocked. However, there were infrequent cases of caffeine-treated spores in which a feature characteristic of rhizoids was expressed, in the absence of asymmetric cell division. The explanatory model proposes that spores have a *pre*-existing stable polarity, such that one end of the spore is differentiated. Previous observation that the direction of outgrowth of the protonema and rhizoid could not be influenced by either unilateral light or plane-polarized light also supports this model (Miller and Greany, 1976).

5. *Higher plant cells*

a) *Eggs*

In higher plant, the zygote or fertilized egg is a highly vacuolated cell suspended in the embryo sac. An initial asymmetric division of the egg defines the polarity which persists throughout the early embryogenesis. The smallest cell, with dense content divides further into the embryo while the larger, vacuolate cell gives rise to the suspensor (Burgess, 1985).

b) *Epidermal cells*

Differentiation of cells in many plant tissues is apparently linked to an *asymmetric* — or unequal — proliferative cell division in the proximal region of the meristem (Barlow, 1984). There are two examples in which the differentiative stimulus is related to the cell's position: trichosclereids which are hair-like cells develop in the cortex of aerial roots such as those of *Monstera*; epidermal root hairs (for their growth, see VI.A.2g). In case of the trichosclereid initials, commitment after signalization may consist of some sort of cytoplasmic polarization, since in many instances the small daughter cell has the more densely-staining (basophily?) cytoplasm. Thus, "differences between the time of commitment and the onset of differentiation can lead to patterned development which may grow if the products of the differential division continue to divide and maintain there the differentiated state" (Barlow, 1984). It is this process which leads to the files of hairs, interspersed by files of hairless cells (Fig. 26 C; Bloch, 1947; Bünning, 1952).

In the leaves of the moss *Sphagnum,* stomata and root hair initials (trichoblasts) as well as chlorophyll-bearing cells can originate by a polar separation of cells with denser protoplasm, probably resulting from a synthesis of new protoplasm (Bünning, 1952). In moss leaves, the apical cell is the first to cease to divide, and it starts to differentiate. This differentiation is associated with cell elongation and the final cell-net depends on the relative amount of elongation of different longitudinal and transverse cell walls (Bopp, 1984). The regular change of division planes results in the equal distribution of daughter cells to either side of the apical cell, forming two symmetrical halves. The descendants of the apical cells produce typical strips of cells (Sych, 1982). In the production of the different types of cells, unequal, asymmetric cell division must be involved and was clearly demonstrated in the leaves of *Sphagnum*. Positional information is necessary for this division pattern as expressed by the postulated gradient of a still unknown substance (Bopp, 1984).

The glandular hair or trichome arises on leaves of *Callitriche* as a polarized extension of one epidermal cell. The extended cell then divides asymmetrically to give rise to a basal cell and a stalk. This raised cell further divides into the multicellular head of the glandular trichome (Burgess, 1985).

The stomatal complex also develops from epidermal cells (Fig. 26 C). Prior to the initiative division, the nucleus of an epidermal cell migrates to an end to give a polarized structure. The asymmetric division gives rise to a small guard mother cell and to a second daughter, vacuolate cell. In its turn this last cell becomes polarized in the same sense as the original epidermal cell; it then divides very asymmetrically. The final developmental stage of the complex is marked by a symmetrical division of the guard mother cell, giving rise to two similar guard cells surrounding the stomatal pore (Bünning, 1957; Burgess, 1985).

In the guard cells of *Allium,* colchicine severely disrupts both the localization of wall deposition and the orientation of new cellulose microfibrils while inhibitors of actin-myosin based microfilament systems do not interfere with normal microfibril orientation in these cells (Palevitz, 1982). Microtubules thus appear to be the major contributors to the microfibrillar orientation in the stomatal complex.

6. *Higher animal cells*

a) *Eggs (animal-vegetal poles)*

If the genome of a differentiating cell varies only in its expression and not in its information content, then we must consider what methods are used by the organism to establish cytoplasmic differences in the cells of the embryo. Apparently this is accomplished by lying down chemically different regions of the egg during oogenesis and having certain regions segregated into specific cells by early cleavage.

The cytoplasm itself of the unfertilized egg is often not homogeneous. Most animal eggs contain stored food material or yolk which, since it is usually concen-

trated in one part of the cell, establishes a distinction between animal and vegetal hemispheres, i.e. it brings about a primary polarization of the cell along the so-called animal-vegetal axis: the *animal* pole is located by the side of polar body formation, while the *vegetal* pole is marked by yolk accumulation settled opposite the egg nucleus.

Mosaic eggs (a^2, etc.) show regional cytoplasmic differences which are so marked that the embryos they produce cannot compensate for missing portions of cytoplasmic materials. *Regulative* eggs (a^5, etc.) give rise to daughter cells capable of developing to complete embryos; the early embryos can regulate their development to compensate for missing portions of cytoplasmic materials. However, both kinds of eggs are difficult to separate (a^1).

A primary polar axiation must be established as an expression of some regional difference(s) in the egg as unravelled by the classical centrifugation experiments of Morgan and Spooner (1909). Their conclusion that the organization responsible for polarity occurs in the stiff outer egg cytoplasm (cortex) was confirmed by the grafting experiments of Dalcq and Pasteels (1937) who proposed the existence of a cortical field. "Morphogenetic substances" are therefore localized in the egg cortex and these substances are probably responsible for the early patterns of differentiation.

In eggs with large amounts of dense yolk such as those of amphibians, the axis of egg polarity approximates the axis of gravity, and the egg floats with its animal pole upward. In eggs with relatively little yolk, such as the sea urchin, the yolk is evenly distributed and the manner in which the egg lies is unrelated to its animal-vegetal axis.

Since polarity is generally not evident in the very early stages of oogenesis, i.e., in the oogonia, it is believed to arise during the growth and differentiation of the oocyte, being imposed on the unpolarized germ cell from the outside (Karp and Berrill, 1981). In oocytes of echinoderms and molluscs, there is a predictable relationship between the position of the oocyte within the ovary and the future animal-vegetal axis. The point at which the oocyte is attached to the ovarian wall, and therefore the point of entrance of supplies to the oocyte from the outside, becomes the vegetal pole. By contrast, in mammals oocytes are completely surrounded by ovarian follicle cells, and there is no obvious relation of any ovarian feature to polarity. As in plants, when an animal egg cell divides, its asymmetrically spread cytoplasmic materials restricted to one or the other pole will be distributed unevenly between the two daughter cells (unequal division), depending on the orientation of the plane of cleavage.

a^1 *Echinoderms (sea urchins)*

The simple but powerful experiments of Morgan and Spooner (1909) on these centrifuged eggs demonstrated that even "regulative" embryos begin development

with an axis of polarity, at least, preformed. The sea urchin egg is therefore *not* isotropic and this leads to think that there are *not* two perfectly separable and distinct kinds of development, "mosaic" and "regulative" (Jeffery and Raff, 1983).

In his monumental text-book, E.B. Wilson (1925) had emphasized that the "morphogenetic determinants" may not be in their final locations at the very start of development. It is by following the fate of a natural egg marker such as the red pigment band in *Paracentrotus lividus* (Morgan, 1927) that it has been possible to know that defined regions of the egg cytoplasm can give rise to particular structures during the course of embryogenesis. Since, architecturally-differentiated domains of the egg cytoplasm have been evidenced by differential organization of the molecular cytoskeleton that match the domains of those morphogenetic "plasms". If one could demonstrate a maternal mRNA localized in the cytoplasm, the specificity for such a localization must lie in the cytoskeleton as well as in the mRNA (Raff, 1983).

a^2 *Molluscs and Worms (Annelids and Nematodes)*

In molluscs and annelid worms, the normal first cleavage partitions critical cytoplasmic constituents asymmetrically and is therefore a *determinate* cleavage; when the daughter cells are separated, they do not have equivalent developmental potentialities. In some molluscs (mussels and sea snails), a protuberance called the polar lobe develops on the fertilized egg cell just before the first cleavage occurs. The plane of the cleavage is oriented in such a way that one of the two daughter cells receives the entire polar lobe. Something in the polar-lobe material must be essential for formation of mesoderm, and its asymmetric distribution makes the first cleavage of the egg a determinate one.

Cytoplasmic localizations are established during oogenesis in the mosaic egg of the nematode *Caenorhabditis elegans* and appear to exert fixed determinative influences after cleavage and parcelling out of cytoplasm (Hirsh, 1979). The first divisions of its zygote are also asymmetric, producing a larger cell at the anterior than at the posterior pole. Subsequent division planes are not orthogonal as in sea urchins and so the arrangement of the embryo cells is more complex (Loomis, 1986).

a^3 *Insects*

Regional differences in morphological as well as chemical properties occur in many eggs along a specific axis — the egg axis. This egg axial polarity subsequently influences development, the anterior pole region of oblong insect eggs normally differentiating to head-thorax segments and the posterior pole to abdominal segments. This *anterior-posterior* polarity, encoded by *bcd* and *nos* genes (see VIII.B.2d[1]), is laid down when the egg of *Drosophila* is formed in the ovary under the control of the maternal genome. The mutation *dicephalic (dic)* affects not only follicle development, thereby altering the antero-posterior polarity of embryonic patterning, but also

the egg shell polarity (Lohs-Schardin, 1982). Other maternal-effect mutants (*dl, snake,* see VIII.B.2d[1]) affect the *dorsoventral* polarity.

Observations of maternal-effect mutants suggest that egg cytoplasm contains substances which define the spatial coordinate of the futur embryo but little is currently known, however, about the substances coded by the maternal genes that endow the egg cytoplasm with its spatial polarity. It is only recently that several mRNAs have been isolated that are localized to either the animal or vegetal hemisphere (Rebagliati *et al.,* 1986). One of these mRNAs, Vg1, has been recently found (Yisraeli and Melton, 1988) to be distributed homogeneously throughout the cytoplasm of early-stage oocytes and localized during oogenesis at their vegetal cortex; translation of this Vg1 mRNA is not required for the localization of the message itself. Thus, «the information necessary to interpret the animal-vegetal polarity in oocytes is present in the naked mRNA transcript» (Yisraeli and Melton, 1988). These last authors make the interesting suggestion that «by specifically interfering with different cytoskeletal elements, and by identifying cytoplasmic components that specifically recognize Vg1 mRNA, it should be possible to detect the localization process and to understand *how* polarity is established and interpreted.»

Primordial germ cell differentiation, first studied in Chrysomelid beetles by Hegner in 1908, is initiated when nuclei arrive at the polar region of the oblong egg and enter a special region of cytoplasm which appears to function as the germ cell determinant (Davidson, 1986). Hegner succeeded in selectively destroying the polar cytoplasm with a hot needle before the peripheral movement of the nuclei had occurred. In the mosaic type of eggs of higher Diptera or of Lepidoptera, the main regions of the body appear to be already mapped out in the cortical plasma at the time of lying, before the zygote has even begun to divide. The entire cortical cytoplasm of the egg is rich in nucleoprotein, but the relative importance of protein and nucleic acid in the early stages of determination is uncertain (von Borstel, 1957).

Suggestively, eggs of *Formica* with a large accumulation of nucleic acids at the hind pole develop into normal females while those with a smaller accumulation become workers (Bier, 1954). In the Cecidomyid midges the germ line nuclei retain their full complement of chromosomes, whereas most of the chromosomes are lost from the future somatic nuclei. But if the somatic nuclei are exposed to the nucleoprotein of the pole plasma the elimination of chromosomes is arrested (Geyer-Duszynska, 1959). It may be that the characteristic effects, which the different regions of the cortical plasma exert upon the nuclei that enter them, depend upon differences in the nature of the RNA content of each zone.

Convincing evidence that causal pole cell determinants are localized in the posterior region of the *Drosophila* egg was provided by UV-irradiation (Geigy, 1931) and by cytoplasmic injection (Illmensee and Mahowald, (1974). The posterior polar cytoplasm can specifically induce pole cell formation and it contains substances necessary for germ cell formation. However, additional demonstrations of the ability

of ectopically induced pole cells to give rise to functional gametes is clearly required to secure the proposal that the polar cytoplasm actually determines the germ cell lineage (Davidson, 1986).

Since the data from eggs of lower insects clearly contradict the mosaic hypothesis, embryonic pattern formation in these forms required other explanations. Seidel (1961) first suggested that pattern formation ensues from a region near the middle of the prospective germ anlage, called the *differentiation center.* According to Seidel's interpretation the immigrating cleavage energid triggers in the polar ooplasm a change of physiological state which propagates anteriorly until it reaches and "activates" the differentiation center. Planning to test the relevance of Seidel's ideas for eggs from another insect group, Sander (1984) performed large series of ligation experiments on leafhopper eggs, varying both site and stage of ligation. If both fragments of the leafhopper egg continue developing after early ligation, the partial germ bands they produce do not add up to the complete pattern: some segments are formed neither in the anterior nor in the posterior fragment, and thus a gap remains somewhere in the middle of the germ band pattern. Details of the gap phenomenon suggested that interaction between posterior and anterior egg regions in the leafhopper (and most other species) is not one-way but mutual, and this leads to the assumption of the second (anterior) gradient.

Hideo Yajima (1964) showed that centrifugation or local UV-irradiation can inflict global changes which lead to "double abdomen" or "double cephalon" patterns instead of a normal larva. The analysis of ooplasmic determinants in Yajima's "anterior formative locality" was carried down to the molecular level by Kalthoff (1983) in an impressive series of experiments on double abdomen induction in chironomids (Fig. 35 C).

A good correlation has been recently found between preliminary physiochemical data and the morphologically confirmed determinants (Schwemmler, 1987): in the ooplasm of unfertilized egg, fermentation activities dominate with an optimum at the posterior pole, upon which the similarly glycolytically active endocytobionts have a direct or indirect influence; in the fertilized egg, the mitochondrial respiration activities gradually increase, beginning in the anterior pole at the cost of glycolysis, and then dominate after egg deposition.

a^4 *Ascidian-Tunicates*

Three different ooplasms, the ectoplasm, the endoplasm, and the myoplasm, are visible in living eggs and sectioned material. They each occupy defined territories in the unfertilized egg. The transparent ectoplasm, derived almost entirely from the sap that escapes from the germinal vesicle (GV) at the time of maturation, is located in the animal hemisphere. The yolky endoplasm surrounds the ectoplasm in the animal hemisphere and almost completely fills the vegetal hemisphere. The myoplasm

occupies the entire cortex of the egg and contains pigment granules which are tinted yellow in *Styela* and orange or red in *Boltenia* (Conklin, 1905).

The unfertilized *Boltenia* oocyte has three major voltage-dependent currents carried through Na^+ and Ca^{2+} channels activated by depolarization, and inwardly rectifying K^+ channels, activated at potentials negative to rest. Electrophysiological changes then occur between fertilization and the eight-cell stage. Lineage specific development of calcium currents have been measured by the whole-cell patch clamp technique during ascidian embryogenesis (Simoncini *et al.*, 1988).

In ascidian eggs, mRNA molecules are tenaciously associated with specific ooplasms as implied by their remarkable resistance to mixing during ooplasmic segregation (Whittaker, 1979; Jeffery, 1984).

a^5 *Amphibians*

During oogenesis, the amphibian egg becomes polarized along its animal/vegetal (A/V) axis (Fig. 36 A). That polarity can easily be seen in the superficial pigmentation pattern of virtually all species of amphibians eggs, which typically are darkly pigmented in the animal hemisphere and lightly pigmented in the vegetal hemisphere (Malacinski, 1984). The pigment polarity is complemented by a polarization of internal components: the smaller, less densely packed yolk platelets in the animal hemisphere, the larger, more densely packed platelets, in the vegetal hemisphere. Around the A/V axis, the egg is radially symmetrical, i.e., cross sections which pass through the A/V axis are identical at virtually all longitudes around the egg's equator.

To explain the origin of the animal-vegetal axis in oogenesis, early embryologists proposed that the native oocyte gains its polarity from the asymmetric environment of its follicle. Gravity was suggested to produce the yolk gradient in amphibian eggs (Pasteels, 1951). However, in general, the arrangement of the internal contents has no relation to the position of the cells in the gravitational field, as particularly shown in the *Drosophila* retinulae (Waddington, 1962). It has been more recently shown that oocytes are not aligned in the follicle relative to the stalk, and are random with respect to the frog's body axis and to the gravitational vector; therefore the notion of the environmental induction of polarity has lost support (Jeffery and Raff, 1983). The alternate idea is that animal-vegetal polarity of the egg originates from prior internal structure such as the nucleus-centrosome axis of the oogonium or earliest oocyte.

As for the difference in pigmentation of the animal-vegetal, dark-light hemispheres respectively, it has been localized in the cortex of the ovocytes in which pigment granules are first uniformly distributed and only later restricted to the dark hemisphere. An asymmetric location of the Golgi system might explain such differential pigmentation. If the Golgi terminus of the cell were a site of active plasma membrane insertion, then a new pigmentless surface might originate from one pole,

while the old and less expanding surface might gradually recede with all the pigment granules (Gerhart *et al.*, 1983; Malacinski, 1984). This proposal would provide a plausible scheme for the genesis of the specialized polarity of the egg from a set of inherent, general, polarized cell functions.

While, during oogenesis in insects (*Drosophila,* see p. 213), the initial cue for establishing the anterior patterning system may by the cellular organization of nurse cell/oocyte complex (MacDonald and Struhl, 1988), in amphibians (*Xenopus,* etc.), initial trancripts become progressively localized within the oocyte, "suggesting that mRNA localization depends on a prior localization of the presumed receptors or anchoring molecules within the egg" (Rebagliati *et al.*, 1986).

Amphibian eggs can be considered as models for A/V polarity. According to Malacinski (1984), it could best be simply defined as "the stratification from animal to vegetal pole of cytoplasmic components which is built up during oogenesis". The significance of the differential distribution of yolk platelets and other inclusions (e.g. germplasm) should, however, not be overemphasized because, even after the cytoplasm organization is perturbed by egg inversion, substantial pattern regulation can ensue. As for the function of that polarity, it could be "for early pattern formation (e.g. cleavage and involution), perhaps simply to ensure a high frequency of orderly development following egg activation". For later pattern specification, A/V polarity could be most significant for primary embryonic induction, that is, for insuring that cells in the animal hemisphere develop the capacity to respond to the action of the primary embryonic organizer (see VIII.B.2f).

A correlation appears to exist between a double gradient of metabolism and the double gradient of differentiation. The causal link between these two phenomena comes from the analysis of the metabolic gradient under conditions that are known to affect the morphogenetic gradients. The two appear to be linked. For example, lithium treatment abolishes the center of metabolic activity at the animal pole without affecting that of the vegetal region. Animalizing agents have the opposite effect and leave the embryo in possession of an intact animal gradient but lacking a counterbalance from the opposite pole. In each case, the ratios of the two influences are greatly disturbed, and the expected abnormal differentiation results.

a[6] *Fishes, Reptiles and Birds*

Yolk platelets take up almost the volume of most of these eggs. Cytoplasm is therefore limited to a relative cap or germinal disk on the top of the yolk. The first two cleavage furrows are orthogonal, but division is incomplete namely the cells are open to the yolk at the bottom (Fig. 8.4. in Loomis, 1986).

a[7] *Mammals*

Their eggs are small with much reduced yolk content. Polar bodies are formed before the first cleavage (Loomis, 1986).

Spermatozoons born from spermatids by the process called spermiogenesis are beautifully constructed polar cells, with their two-part head (nucleus and acid acrosome) and the mitochondria in the midpiece supplying the ATP required for the motility of the back tail (Karp and Berrill, 1981).

Early mouse embryogenesis is not only of the regulatory but also of the mosaic type. The process of differential inheritance is of crucial importance in generating cell diversity in the early mouse embryo. According to Johnson and Pratt (1983), both induction of polarity and the polarizing response are observed at the normal developmental time in embryos placed in an effective dose of α-amanitin at the late two-cell stage even though cleavage is blocked. Thus, any mRNAs required for the events of polarization must be present by this time. Whether these mRNAs are maternal or newly transcribed and embryonic is still unknown.

From oocyte to early eight-cell stage there is *pre*polar organization of membrane and subcortical cytoskeleton which is altered in a reversible and contact-dependent manner. By the eight-cell stage, the epithelial axis is established and this organizing activity is expressed by its stabilizing effect on the cytoskeleton evidenced by the apical polarization of the actin and tubulin cytoskeletons that in turn mobilize cell organelles (Fleming and Johnson, 1988).

b) *Epithelia (apical-basolateral poles)*

The ability of epithelial membranes to function vectorially depends on the polarity of the epithelial cells, observable both in the asymmetric distribution of organelles in the cytoplasm and in the segregation of different sets of proteins into two opposite plasma membrane domains, apical (luminal) and basolateral, separated by tight junctions (Sabatini *et al.*, 1983). The basal surface of the basolateral membrane is that which faces the blood supply on the serosal side, while the apical surface faces the luminal side (Fig. 27).

Epithelial cell lines can retain in culture polarity properties of natural epithelia and confluent monolayers of canine kidney cells become morphologically and functionally polarized. Like natural epithelia, canine kidney cells generate two domains in the plasma membrane, apical and basolateral, separated by tight junctions. Tight junctions are thought to be essential for maintaining the functional polarization of epithelial cells by preventing the intermixing of plasma membrane components, which must be segregated exclusively to one or the other cell surface domains. Tight junction associated-proteins have been recently isolated and cingulin characterized as an acidic highly elongated, peripheral component (Citi *et al.*, 1988).

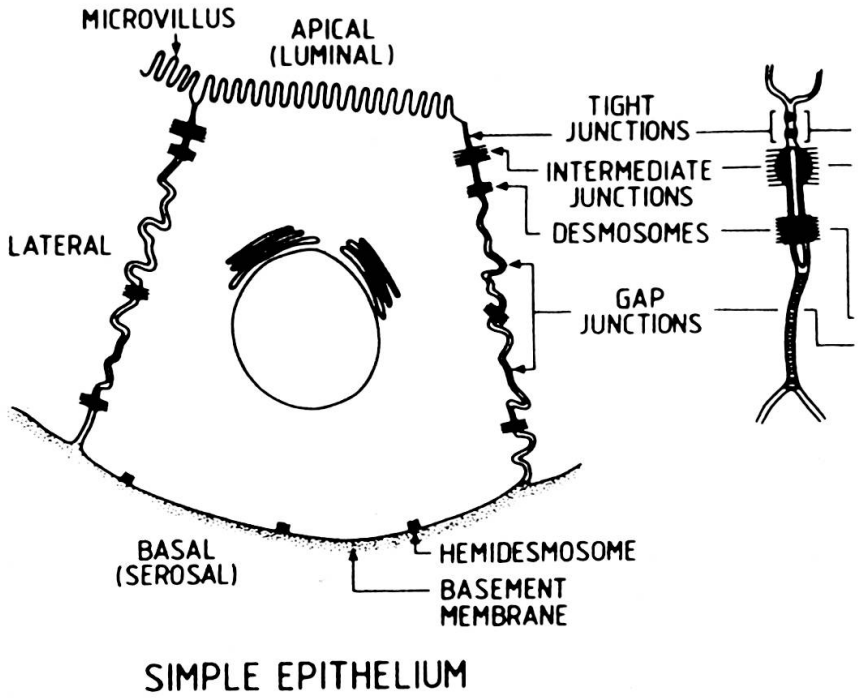

FIG. 27.

Working model depicting the organization and major proteins of the brush border cytoskeleton of a simple epithelium: apical (luminal)— basal (serosal) polarity. From M. S. Mooseker, 1985. Organization, chemistry, and assembly of the cytoskeletal apparatus of the intestinal brush border. Ann. Rev. Cell Biol. 1: 209-241, with authorization from Annual Reviews Inc., Palo Alto, U.S.A.

The two surfaces of apical and basolateral domains are characterized by markedly different protein compositions, reflecting the ability of the cell to target newly synthesized membrane proteins to specific regions of the cell surface (Simons and Fuller, 1985; Matlin, 1986). This targeting capability is also apparent in the polarized release of secretory products, distinct sets of secretory proteins being released from their apical and basolateral poles (Kondor-Koch *et al.*, 1985; Gottlieb *et al.*, 1986; Gonzalez *et al.*, 1987; Roth *et al.*, 1987). The fundamental characteristics that allow this vectorial transport across an epithelial cell are the differential sorting and insertion of transport proteins either in apical or basolateral plasma membrane, and the preferential association of endocytosis and exocytosis with one or the other pole of the cell. That plasma membrane proteins are asymmetrically distributed between the apical and basolateral surface is additionally demonstrated by the asymmetric budding of enveloped viruses from only one plasma membrane domain (Rodriguez-Boulan and Pendergast, 1980).

According to Caplan *et al.* (1987), secretion of basement membrane components (heparan sulphate proteoglycan (HSPG) and laminin) takes place from the basolateral cell surface and this polarized release results from active sorting. The

sorting process which mediates this polarized secretion requires an acidic intracellular compartment. MDCK cells treated with NH_4Cl to raise the pH of their intracellular compartments secrete laminin and HSPG by a default pathway which leads to their release in roughly equal quantities into the medium of both the apical and basolateral compartments. The nature of this acidic compartment and its role in sorting remain unknown. It is tempting to postulate that the sorting of basolateral secretory proteins (that is basement membrane components) occurs by mechanisms similar to those that mediate diversion of newly synthesized lysosomal enzymes to lysosomes (Caplan *et al.*, 1987).

Polarization of plasma membrane domains is also an essential feature of secretory epithelial cells from exocrine glands. The surface of exocrine cells (a typical example is the acinar cell of the pancreas) is separated into an apical domain, where secretion occurs by exocytosis, and a basolateral domain, which senses variations of the internal milieu and is enriched with receptors for various hormones and secretagogues. It is unknown, for example, whether hormone release occurs through the same region of the cell surface that receives exogenous stimuli or whether endocrine cells possess distinct "sensitive" and "effector" poles. The data presented by Lombardi *et al.* (1985) clearly demonstrate polarization of plasma membrane domains in cultured pancreatic endocrine cells and provide the basis of future efforts to unmask plasma membrane polarization in endocrine cells *in vivo*. In this respect, it is worth mentioning that recent physiological data are consistent with the possibility that distinct venous (receptor-rich) and arterial (secretory) poles exist in pancreatic insulin-producing cells.

Vectorial solute transport by epithelia requires the polarized insertion of transport proteins into apical or basolateral plasmalemmal domains. In the specialized intercalated cells of the kidney collecting duct, the selective placement of an apical plasma membrane proton-pumping ATPase (H^+-ATPase) and of a basolateral membrane anion-exchange protein results in transepithelial proton secretion. Recently, it was proposed that intercalated cells can reverse their direction of proton secretion under different acid-base conditions by redirecting proton pumps from apical to basolateral membranes, and anion exchangers from basolateral to apical membranes (Schwartz *et al.*, 1985). But others have found that antibodies raised against the red cell anion exchange protein only labelled intercalated cells at the basolateral plasma membrane, providing evidence against the model of polarity reversal. Brown *et al.* (1988) have found that some cortical collecting duct intercalated cells have apical plasma membrane proton pumps, whereas others have basolateral pumps. This is the first direct demonstration of neighbouring epithelial cells maintaining opposite polarities of a transport protein. Thus, either subtle structural differences exist between proton pumps located at opposite poles of the cells, or factors other than protein sequence determine the polarity of H^+-ATPase insertion.

The interaction between membrane proteins and cytoplasmic structural proteins is thought to be one mechanism for maintaining the spatial order of proteins within functional domains on the plasma membrane (Singer, 1974). In polarized epithelial cells homologues of ankyrin and spectrin (fodrin) are localized in specific membrane domains. Nelson and Veshnock (1987) showed that ankyrin binds to the ubiquitous $(Na^+ + K^+)$ATPase, which has an asymmetrical distribution in polarized cells (Hootman, 1986). Changes in the cellular organization of fodrin result in the formation of a highly insoluble, relatively dense and stable layer of that structural protein which appears to be localized to the cell periphery and predominantly in the region of the basolateral plasma membrane of canine kidney epithelial cells in continuous monolayers (Nelson and Veshnock, 1986). The formation of this structure coincides temporally and spatially with extensive cell-cell contact, and with the development of the polarized distribution of the Na^+, K^+-ATPase, a marker protein of the basolateral plasma membrane.

The cylindrical hair cells of the inner ear are also apico-basally differentiated cells of epithelial origin (Roberts *et al.*, 1988). They are receptors which act as extremely sensitive mechano-electric transducers; they convert a mechanical force, the stimulus applied to the hair bundle, into an electric signal, the message relayed to the brain. The hair bundle is arranged with a plane of symmetry and all but one (kinocilium) of its extensions are called stereocilia. In the cell itself, structural polarity is evidenced by the exclusion of mitochondria from the apical, cuticular plate zone and the afferent nerve ending at the basal pole surface. Both the apical and the basal surfaces of the hair cell act as capacitors thin plates separating electric charges with opposite signs and both areas have channels for ions. In its directional sensitivity, the hair cell is capable of resolving any stimulus into two components: a first, along the axis of bilateral symmetry, to which the cell responds with a depolarization (one directional push) or a hyperpolarization (push in the opposite direction). To the second component, along the perpendicular axis, there is no response.