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VI. POLAR CELL GROWTH

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

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

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

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

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

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

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

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

A. MONOPOLAR

1. Outgrowth (emergence)

a) *Spherical buds*

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

a' *Bacterial budding*

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

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

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

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

a[2] *Yeast budding*

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

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

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

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

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

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

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

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

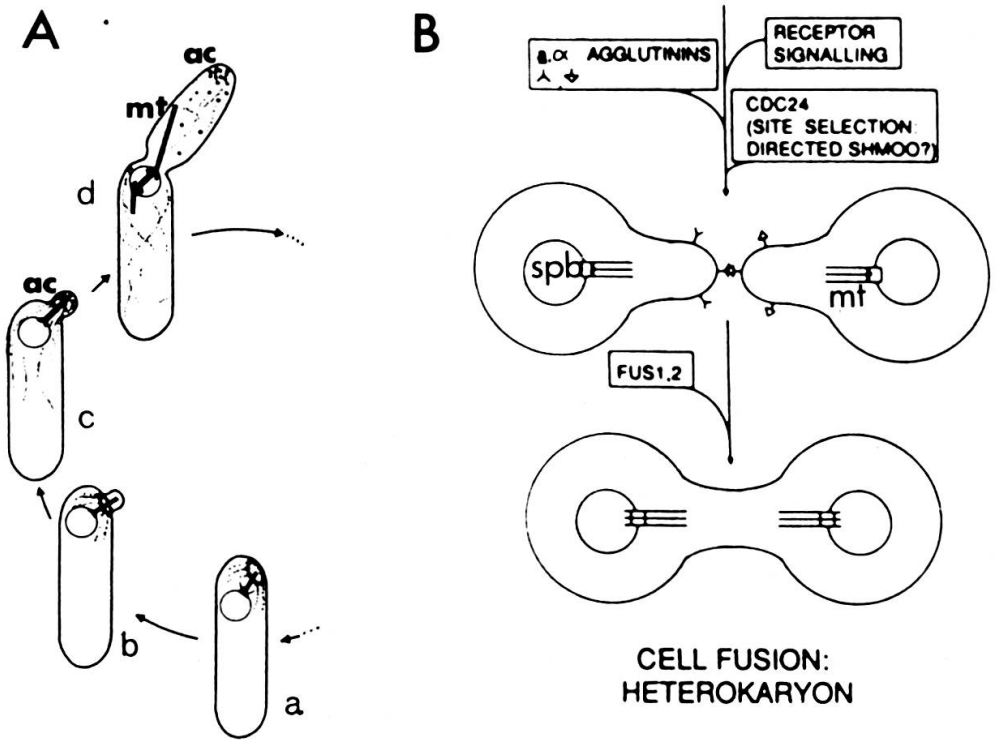

FIG. 18.

Monopolar budding of yeast.

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

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

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

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

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

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

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

b) *Cylindrical germ tubes*

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

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

b' *Fungal spores*

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

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

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

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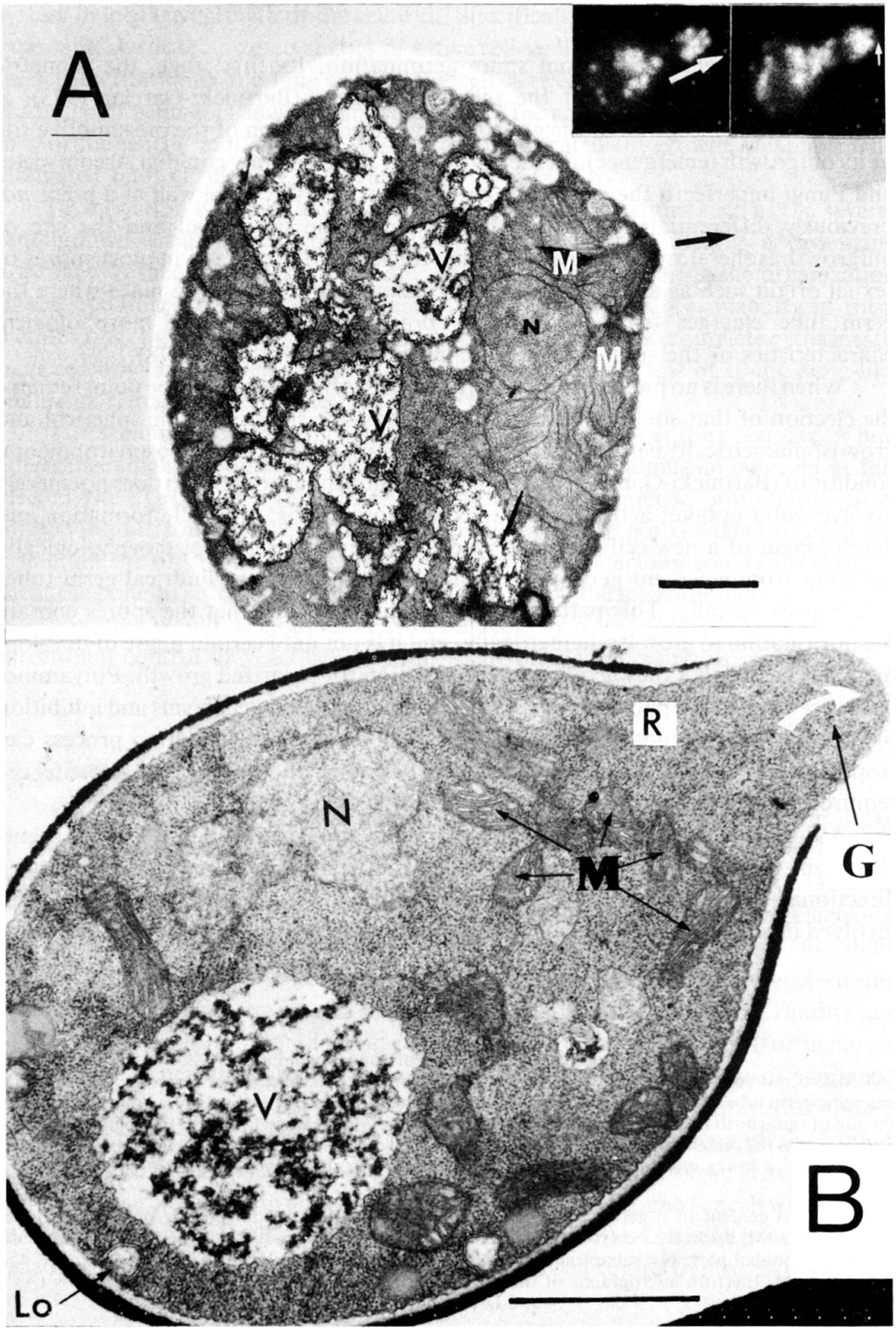
FIG. 19.

Monopolar germination of fungal conidia.

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

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

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


A
V
M
N
M
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R
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G
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B
Lo

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

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

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

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

b² *Algal eggs*

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

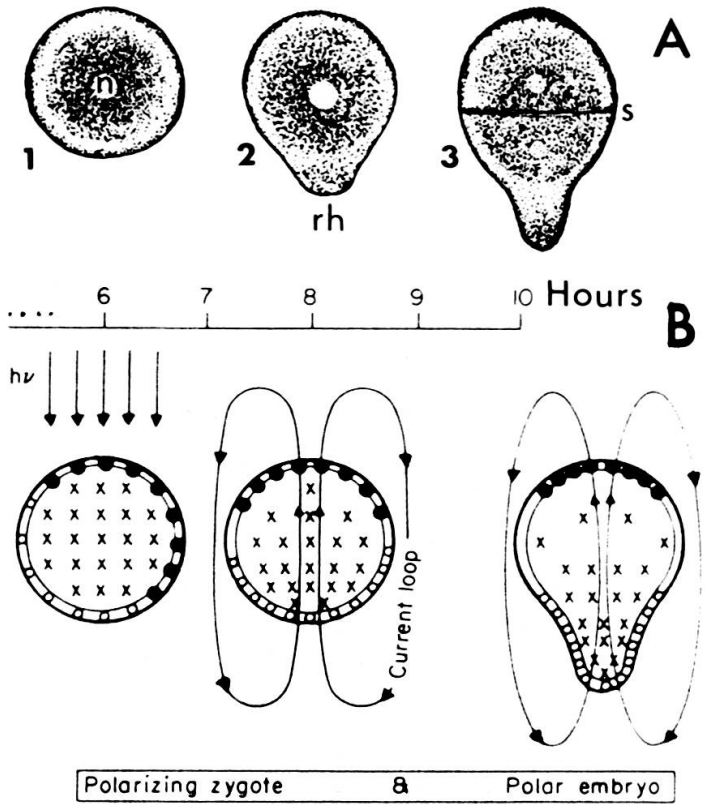

FIG. 20.

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

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

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

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

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

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

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

b^3 *Moss and fern spores*

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

$b^{3'}$ *Mosses*

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

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

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

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

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

b[4] *Pollen grains*

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

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

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

c) *Spherical/cylindrical alternative = dimorphism*

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

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

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

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

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

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

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

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

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

2. Tip growth

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

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

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

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

a) *Prokaryotic microhyphae*

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

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

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

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

b) *Fungal hyphae*

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

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

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

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

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

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

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

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

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

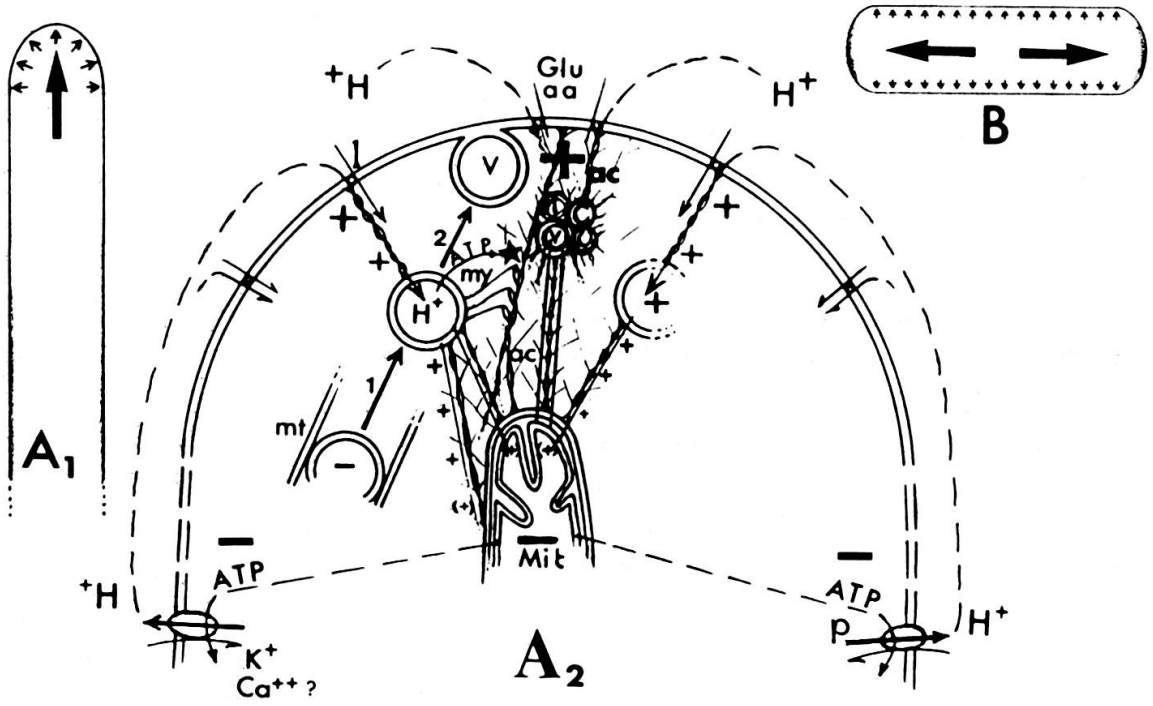

FIG. 21.

Models of polar growth.

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

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

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

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

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

c) Algal rhizoids and filaments

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

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

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

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

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

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

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

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

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

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

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

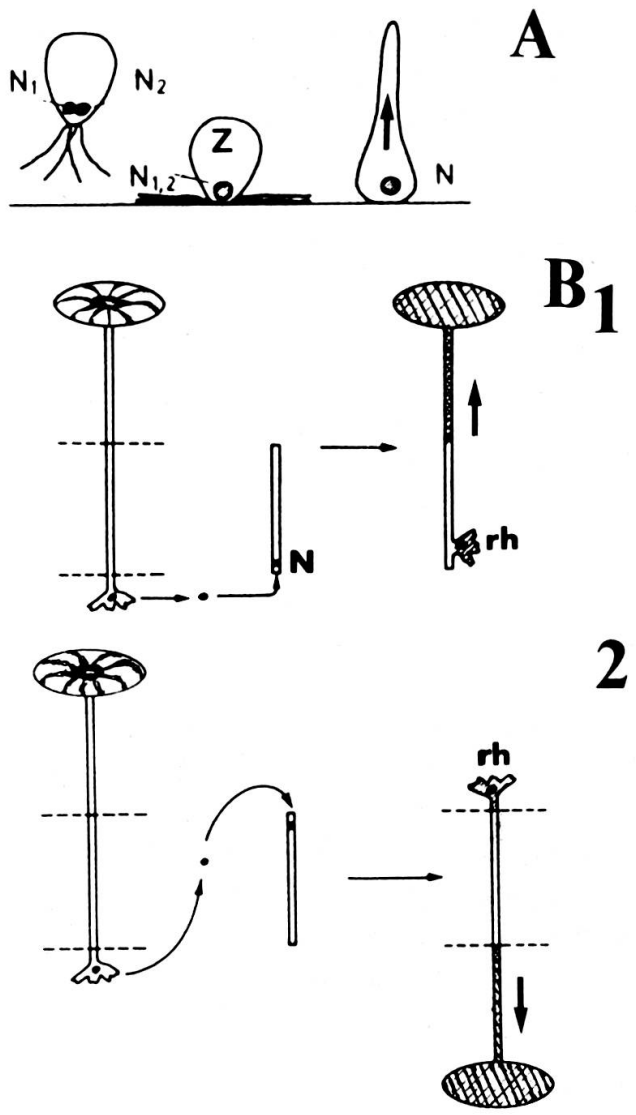

FIG. 22.
Polarity control in *Acetabularia.*

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

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

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

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

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

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

d) *Protonema (mosses)*

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

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

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

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

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

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

e) *Prothallia (primary fern stage)*

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

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

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

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

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

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

f) *Pollen tubes*

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

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

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

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

g) *Root hairs*

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

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

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

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

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

h) *Insect bristles*

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

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

i) *Animal neurites*

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

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

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

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

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

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

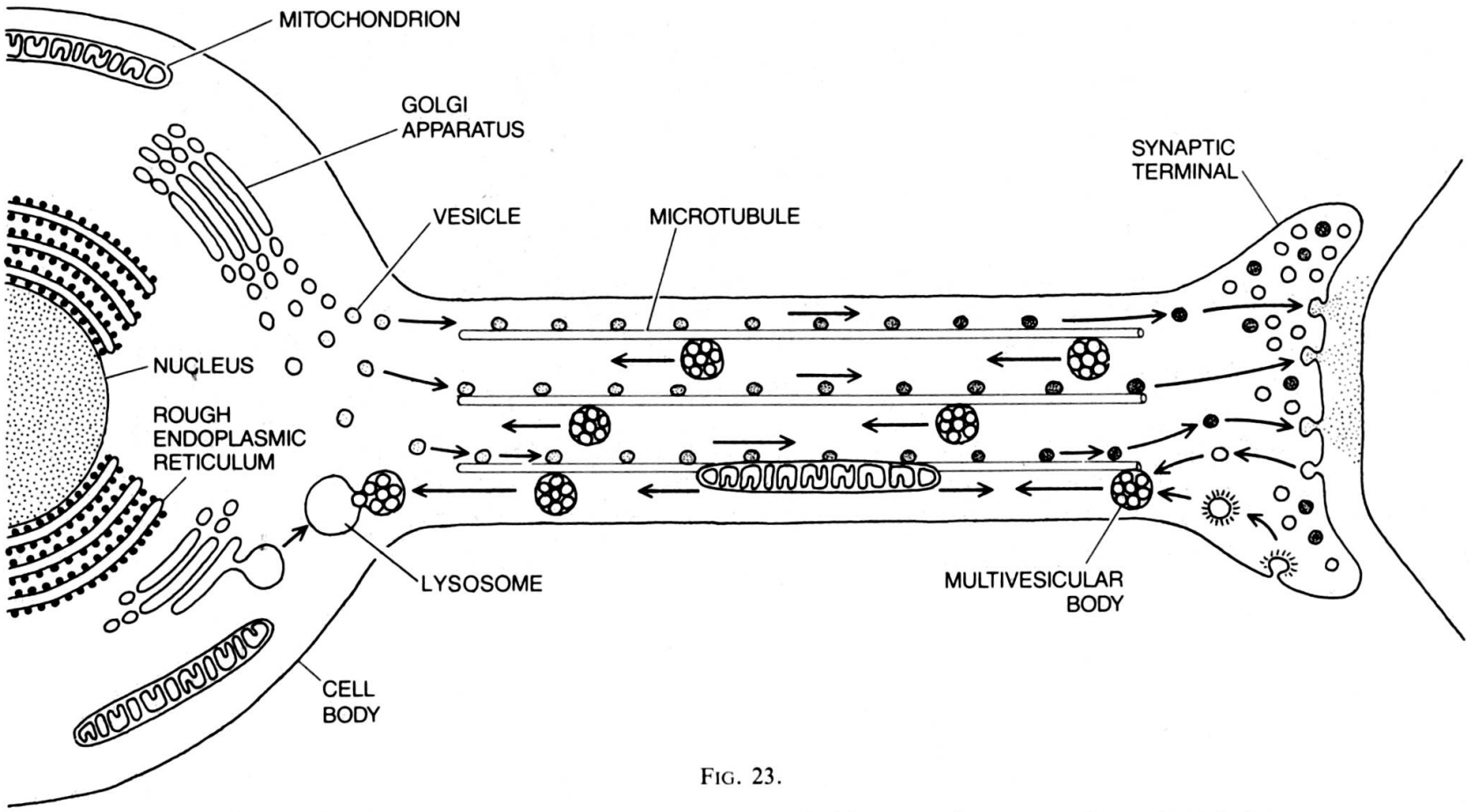

FIG. 23.

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

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

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

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

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

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

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

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

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

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

B. BIPOLAR GROWTH (EQUIPOLAR, HOMOPOLAR)

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

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

a) *Bacterial elongation*

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

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

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

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

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

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

b) *Yeast elongation*

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

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

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

c) *Yeast double budding*

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

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

d) *Fungal double germ tubes*

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

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

e) *Algal cells elongation*

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

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

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

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

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

f) *Higher plant elongating cells*

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

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

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

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

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

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

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

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

C. MULTIPOLAR

a) *Apiculate yeast buddings*

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

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

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

b) *Fungal germ "multitubes"*

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

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

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

c) *Desmidial algae (multiradiate pattern)*

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

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