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VI. POLAR CELL GROWTH

A. MONOPOLAR

1. OUTGROWTH (EMERGENCE)

a^2 *Yeast budding*

Budding in the yeast *Saccharomyces cerevisiae* involves a polarized deposition of new cell surface material that correlates with a highly asymmetric deposition of the actin cytoskeleton. In cell cycle deficient mutants, this cell-surface deposition is delocalized and found to be associated with a loss of asymmetry (?) of the actin cytoskeleton (Adams *et al.*, 1990). In the control of the budding direction of CDC mutants of *Saccharomyces cerevisiae* it has been shown by Matsuoka *et al.* (1988) that it is the stage after spindle pole body duplication which is effective for that directionality using an electric stimulus.

The events associated with budding and those of the nuclear cycle thus represent two independent pathways within the cell cycle. These new results are supported by the fact that temperature sensitive mutant cells (CDC42 and 43) arrest as large unbudded cells when grown at restrictive temperature while events associated with the nuclear cycle (DNA replication and formation-elongation of mitotic spindle) continue. The mutant CDC42 which is involved in the development of yeast cell polarity has been molecularly characterized (Johnson and Pringle, 1990). Some of its proteins have been found to show similarities to *ras* proteins and some of these similarities were more pronounced in the regions that have been implicated in GTP binding and hydrolysis, thereby suggesting membrane and/or microtubules involvement in CDC42 function. The CDC43 gene product also involved in establishing cell polarity during the yeast-division cycle has recently been isolated and its sequence analyzed (Johnson *et al.*, 1990).

b^1 *Fungal germ tubes*

In a study of the inductive signal to shift from polarized germ tube growth to non-polarized appressorial growth in *Metarhizium anisopliae*, St Leger *et al.* (1990) found "no evidence for a gradient of Ca^{2+} in the spore which could establish the initial polarity. Calmodulin, however, was localized at the poles of the conidia, near the site of germ-tube emergence".

b^2 *Algal eggs*

Polarization of the zygote in Fucoid algae leads to its asymmetric cleavage on germination (see **I**). The equal first division of the zygote corresponds to its asymmetric

cleavage. This cytological process is preceded by a polarization of the initially spherical cell which can be equated to the symmetry-breaking defined by Prigogine's school (Prigogine and Stengers, 1984). It involves polarization of the whole cell, from its peripheral wall to its cytoplasmic content along an antero (thallus)- posterior (rhizoid) axis of polarity. The polarizing role of light and external ionic gradients has already been emphasized (see **I** and **II**). It should be noted that Kropf's (1989) experiments with fluorescent chelators have not supported an interaction between these two inductors.

c) *Dimorphism*

pH appears to control the dimorphic choice between sexual process, namely macrocyst formation and asexual development, namely sorocarp formation (Iijima and Maeda, 1990).

2. Tip growth

Its processes have been recently reviewed not only in fungal but also in plant cells (Heath, 1990).

b) *Fungal hyphae*

In 1982, Koch has developed a formula to account for the shape of the apex of hypha and allowing ''the calculation of the rate of synthesis at a point on the tip from its distance from the axis and the slope of the tip at that point''. Koch considered the growing hyphal tip as ''analogous to a molten glass bubble blown under special conditions''.

Apical growth in fungi and other developmental processes in yeasts and algae have recently been shown to illustrate the diversity of vectorial physiology and of the forms it generates (Harold, 1990). In such processes, according to Turian and Favre (1990), ''the primary polarity signal in a relatively isotropic structure such as the conidium of *Neurospora* would be endogenously endorsed by a few uncoupled mitochondria strategically positioned by microtubules to enforce, by protons from their dissipative gradient, electrical depolarization of a local site of the plasmalemma, thereby selected as the genetically determined single outgrowth site''. By contrast, germinated conidia of *Monilia fructigena* are bipolarly determined (Turian, 1985, see **I**). The local lowering of pH would be an acid activation signal for phospholipase expected to hydrolyse phospholipids to unsaturated agents uncoupling frontal mitochondria, thereby lowering their ATP productivity and favoring inward leakage of H^+ with the electrical loops entering into the elongating hyphal tips. Moreover, phospholipase activation would act as a secondary signal for production of inositol 1,4,5-trisphosphate-mobilizing Ca^{2+} from its endomembrane store and thus concour to set a concentration gradient of calcium ions in the hyphal apices (Turian and Favre, 1990). Concerning this problem of apical growth it

must be pointed out that recent results from Harold's group disprove any obligatory connection between inward current and hyphal outgrowing processes. Consequently the hypothesis that electric currents localize hyphal growth has become questionable and in need of a reassessment (Cho *et al.*, 1991a). This leaves open our above and previous suggestions of an internal control of this process (Turian, 1983, see **I**).

Wild type hyphae of *Neurospora crassa* grown in the presence of the calcium-channel blocker verapamil, unlike untreated controls, do not show Ca^{2+} in their hyphal tips and display an enhanced branching of their hyphae. This hyperbranching mycelial pattern could be corrected by the addition of Ca^{2+}. Two morphological hyperbranched mutants, ''frost'' and ''spray'', failed to demonstrate Ca^{2+} in hyphal tips and could also be corrected to normal branching in the presence of exogenous Ca^{2+} (Dicker and Turian, 1990). These new results support our previous finding of the inverse H^+/Ca^{2+} gradients in the hyphal apex of *N. crassa* (Turian *et al.*, 1985, see **I**). In their controlling role of polar growth, exogenous Ca^{2+} ions would enter through the tips by antiport with the H^+ ions effluxed from subapical mitochondria thereby uncoupled by the Ca^{2+} that they sequester (Turian, 1979, see **I**). This would thus provide an autoregulative system (flip flop mechanism ?) of polarity maintenance.

The ''Spitzenkörper'' which is an aggregate of apical vesicles is centrally positioned at the extreme hyphal apex of septomycetous (not ''Streptomycetous'' as wrongly spelled p. 164 in **I**) fungi. Its disruption by a demethylase inhibitor, cyproconazole, leads to an inhibition of apical extension of the hyphal cell (Roberson and Fuller, 1990).

The hyphae in expanding mushrooms of the *Agaricus* type have been shown to grow by diffuse extension over their whole wall surface (Mol and Wessels, 1990) in contrast to the prevalent apical elongation occurring in colonizing substrate hyphae.

The architecture of the actin and tubulin cytoskeletons of the oomycete fungus *Phytophthora infestans* might be involved in the maintenance of the spatial organization of its hyphal protoplast (Temperli *et al.*, 1990).

c) *Algal rhizoids and filaments*

An abundance of endoplasmic reticulum and Golgi vesicles aggregated into a spherical apical body or ''Spitzenkörper'' have been observed in the tip of fast growing *Chara* rhizoids (Bartnik and Sievers, 1988).

A negative apico-basal gradient has been demonstrated by the fluorescence of chlorotetracycline, a calcium chelator in the growing phase of *Acetabularia* (Reiss and Herth, 1979). This calcium gradient correlates with electrical polarity in growing cells: the apex is hyperpolarized and action potentials due to a chloride pump arise from it (Gradmann, 1976; 1989 in **II**) until the cap is initiated. Calmodulin fluorescence followed the same negative apico-basal gradient as calcium (Cotton and Vanden Driessche, 1987 in Vanden Driessche, 1990). A light sensitive, transcellular current has been detected along the regenerated enucleated posterior stalk of A. *mediterranea* (Novak and Sironval, 1976).

d) *Protonema (mosses)*

In protonemal tip growth of the moss *Physcomitrella patens* microtubules impose polarity and directionality upon expansion while F-actin is necessary for outgrowth (Doonan *et al.*, 1988) as also found in fungi (Turian *et al.*, 1985; Yokoyama *et al.*, 1990).

f) *Pollen tubes*

Shortly before their germination, pollen grains show a precise spatial and temporal organization of actin (Tiwari and Polito, 1988) which has microfilamentous arrays preferentially accumulated beneath germination apertures. Cytochalasin D treatments indicate that an uninterrupted progression of actin organization is essential for pollen germination (Tiwari and Polito, 1990a).

An actomyosin is involved in the transport of organelles and also of larger inclusions in pollen tubes (Heslop-Harrison and Heslop-Harrison, 1989). A concentration of small microtubules, forming a collar at the base of the tube protruding from a germinating pear pollen grain, has been detected by Tiwari and Polito (1990b).

Ca^{2+} appears to play a fundamental role in establishing the dominance of apical growth in pollen tubes (Picton and Steer, 1982, 1983) as it does in fungi (ref. in **I**; St Leger *et al.*, 1990).

i) *Animal neurites*

Fast axoplasmic transport within the squid giant axon provides an excellent model system for viewing directly the bidirectional vesicle transport along "transport filaments" (Allen *et al.*, 1982). Sequential examination in the light and electron microscope revealed that a "transport filament" was a single microtubule without associated actin (Schnapp *et al.*, 1985). An assay system using centrosomal microtubule arrays was used to determine the direction of kinesin movement relative to microtubule polarity, which is defined by the direction of preferred microtubule polymerization (Vale *et al.*, 1985). Within axons the polarity of microtubules is such that the plus (+) ends are oriented toward the synapse (Burton and Paige, 1981; Heidemann *et al.*, 1981). The minus (-) end motile factor (dynein) would be responsible for moving organelles in the retrograde direction, whereas organelle movements in the anterograde direction from the cell body to the synapse would be driven by kinesin (see IV.E.3 in **I**).

Microtubules are polarly oriented in the axons and dendrites in hippocampal neurons. In axons, they are uniformly oriented with respect to polarity: (+) ends are directed away from the cell body toward the growth cone, in sharp contrast with the nonuniform orientation of microtubules in the mid-region of the dendrites. However, at distance within 15 μm of the growth cone, microtubule polarity orientation is similar in both dendrites and axons (Baas *et al.*, 1988).

The neurons in the isthmo-optic brain nucleus are already polarized during the early chick embryogenesis and most neurons near the border of the nucleus already have inwardly polarized dendrites (Clarke and Caranzano, 1985). Dendritic geometry and its modulation in the developing brain involves an early target removal which eliminates a retrograde signal that normally enhances dendritic polarization. In this work on arborization of dendrites and its possible control by the axonal target region, Blaser *et al.* (1990) propose an ''index of polarization'' defined as the ratio between the ''length of polarization'', which is a measure of dendritic polarization, on the square root of the area covered by the dendrites.

In the developing neural tube the flow plate would act as an intrinsic organizer to establish pattern and polarity in this system (Hirano *et al.*, 1991).

j) *Capillary vessels*

Normally well-polarized capillary-like tubes can be recovered from the spherical hemiangioma, aberrant morphogenetic behavior of endothelial cells by neutralization of excess proteolytic activity by exogeneously added serine protease inhibitors (Montesano *et al.*, 1990). The local gradient of angiogenetic rounding of endothelial cells (neovascularization) can be inhibited by antibiotics (Ingber *et al.*, 1990).

In coupled endothelial cells, the electric signal of hyperpolarizing substances can be preferentially directed by the endothelium along the longitudinal axis of arteries (Bény and Gribi, 1989).

B. Bipolar growth

b) *Yeast elongation*

A protein kinase (*kin1*) mutant of the fission yeast grows as spheres on enriched medium in contrast to the wild type cells, which grow as rods. The gene *kin1*-positive is therefore important for growth polarity in *Schizosaccharomyces pombe* (Levin and Bishop, 1990). As in *S. pombe*, F-actin was found in *S. japonicus* to be concentrated as "dots" at the growing poles of interphase cells (Alfa and Hyams, 1990).

e) *Algal cells elongation*

The response of round *Euglena gracilis* cells to Ca^{2+} channel blockers in the early part of their light cycle suggested that the process of gradual cell elongation produced was the result of an uncoupling from the biological clock (Lonergan, 1990). *Euglena* cells have been studied by the detergent-extracted cell model which has been adapted by Lonergan (1990) to the study of microtubule repolymerization.

f) *Higher plant elongating cells*

The acid growth theory of auxin action has been recently reexamined by Böttger's group who concluded from their experiments using a computer-controlled pH-stat that "'the acid growth theory correctly describes incidents taking place in the early phases of auxin-induced growth'' (Lüthen *et al.*, 1990).