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Autor: Turian, Gilbert
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VI. POLAR CELL GROWTH

Tropisms are biopolarizing processes which contribute to orient both growth and morphogenesis. Some of these processes such as galvanotropism and magnetotropic responses are more directly relevant to monopolar growth: fungal hyphae (A.b), pollen tubes (A.f), neurites (A.i), etc. Others such as gravi- and phototropisms are rather implicated in morphogenetic polarizations (tropic curvatures in higher plants, VIII.A.c[4]).

A. Monopolar

1. Outgrowth (emergence)

a[2] *Yeast budding*

The development of cell polarity in budding yeast involves virtually every aspect of cell biology. Two of its central challenges are the identification of interdependencies between the growth events and the determination of the primary inductive processes (Drubin, 1991). The asymmetry of yeast growth is also reflected in the rearrangement of many organelles (Baba *et al.*, 1989) and of the cytoskeleton (Barnes *et al.*, 1990) thereby polarly organizing the cytoplasm during projection formation ("shmoo" Fig. 18, in **I**) for the mating.

Genes responsible for cell polarity and the axis of cell division in *Saccharomyces cerevisiae* have now been identified as reviewed by Drubin (1991). Such patterns of oriented division involve a polarization of cell surface growth, bud sites being chosen in two distinct spatial patterns (see Hartwell, 1991): *axial* for a or α cells and *bipolar* for a/α cells. A model for bud site selection has consequently been proposed by Chant and Herskowitz (1991) which involves: (1) recognition of a morphogenetic landmark by certain *BUD* gene products; in the absence of *BUD* genes there is a random distribution of the bud sites around the yeast cells. (2) Recruitment of products (*CDC24* gene products) that restrict growth to the bud site; mutants defective in gene *CDC24* are unable to bud or establish cell polarity (Adams *et al.*, 1990 in **III**). (3) Recruitment of products such as actin filaments and secretion machinery necessary for bud growth itself. Moreover, it has been shown that *BUD5* interacts functionally with a gene, *BEM1*, that is required for bud formation (Chant *et al.*, 1991). As expressed by Chenevert *et al.* (1992) "Cell polarization requires that a cellular axis or cell-surface site be chosen and that the cytoskeleton be organized with respect to it". Their finding of the gene *BEM1* necessary for yeast cell polarization and whose product contains two SH3 domains provides such a link between the cytoskeleton and morphogenetic determinants on the cell surface.

Conditional-lethal actin mutants tend to enlarge uniformly instead of polarly localize their growth to a bud (Barnes *et al.*, 1990). Other mutants, lacking the Sac6 protein - a yeast fimbrin homologue - do not form normal actin structures and are also defective in morphogenesis (Adams *et al.*, 1991).

Yeast calmodulin was shown to be localized to sites of cell growth in *S. cerevisiae* but the fact the *cdc24* mutant which has a defect in bud assembly fails to exhibit polarized localization of calmodulin would indicate that the CDC24 gene product is responsible for controlling the polarity of calmodulin (Sun *et al.*, 1992).

The consequences on yeast budding of defects in F-actin structures, especially those resulting of mutations in the actin gene *ACT1* (Novick *et al.*, 1989), have been further reviewed by Solomon (1991). An act2 protein might have an important role in cytoskeletal reorganization during the yeast cell cycle (Schwob and Martin, 1992). The product of the gene *CDC11* and actin both localize to the budding site of yeast well in advance of bud emergence (Ford and Pringle, 1991). As for the vectorial transport of secretory vesicles to the site of bud yeast development, it might be mediated by the *MYO2* myosin gene along actin cables (Johnston *et al.*, 1991).

The polarized protein SPA2 (spindle pole antigen) is also known to accumulate at the sites of yeast vegetative buds and elongating "shmoo" buds (Snyder, 1989, see **II**). Now, G1 cells have been shown to contain a polarized distribution of both a SPA2 protein localized to a patch at the presumptive bud site of cells and a polarized distribution of actin spots in the same region (Snyder *et al.*, 1991b). In that case, the "cytokinesis tag" proposed by these authors would explain the localization process as a *non*-random positioning of bud sites in haploid cells. Rsr1p, a ras-like gene homologous to Krev-1, functions only in bud site selection and not in subsequent events of polarity establishment and bud formation (Ruggieri *et al.*, 1992).

Novel yeast genes can prevent spindle pole body duplication and lead to formation of a monopolar spindle (MPS1 and MPS2 genes) thereby causing monopolar mitosis (Winey *et al.*, 1991). When starved for nitrogen, RAS regulated a/α diploid cells of *S. cerevisiae* undergo a dimorphic transition (c) to pseudohyphal growth which requires a polar budding pattern by unipolar cell divisions (Gimeno *et al.*, 1992).

b) *Fungal germ tubes*

By contrast with axiation-needed microtubules, actin microfilaments are required for germ tube outgrowth from conidia of *Neurospora crassa* (Barja *et al.*, 1991a).

c) *Dimorphism*

Its controlling factors in *Mucor* have been reviewed by Orlowski (1991). In studies on polarization induction by electric fields in another Mucorale, *Mycotypha africana*, addition of calcium has been found to favour the M-form and to reduce the number of budding Y-germlings (Wittekindt *et al.*, 1989, 1990). In nutritional starvation, yeast cells can also undergo a dimorphic transition (see a^2) involving a Congo red-highly

stainable, β-glucan thickening of their intercellular walls (Turian, 1982, unpublished observations).

An alternative of the cell types, coccoid versus filamentous, is also examplified by certain algae, not only *Pleurococcus vulgaris* (see Sinnott, 1960 in **I**) but also by *Pleurastrum* species (Ettl, 1988).

2. Tip growth

b) *Fungal hyphae*

The tip acidification previously described (see **I**) has now also been probed by the quenched fluorescence of acridine orange in the apices of germ tubes outgrown from zoospores of *Saprolegnia parasitica* (Turian *et al.*, 1991). Ca^{2+} ions have also been assigned a role in the polarized apical extension of hyphae (see **I**) and their sequestration in mitochondria fronting the tip of germ tubes outgrowing from conidia of *Neurospora crassa* has been confirmed by chlorotetracycline (CTC) fluorescent probing (Barja and Turian, 1992). Such feature of the hyphal tip thus differs from previous observations on some pollen tubes (see Herth *et al.*, 1990). The absence of CTC fluorescence in the extreme hyphal tip where wall-vesicle membrane concentration and growth associated Ca^{2+} influxes are maxima have also been highlighted by Yuan and Heath (1991). Calcium has also been involved in the regulation of hyphal extension and branching in *Fusarium* species (Robson *et al.*, 1991) which show an increased branching in the presence of calmodulin antagonists as previously shown in *N. crassa* by Ortega Perez and Turian (1987).

The actin concentration detected at the hyphal tips and at the sites of septal formation suggests that "actin may play a central role in tip growth and hyphal wall formation" (Raudaskoski *et al.*, 1988, 1991; Jackson and Heath, 1989; Barja *et al.*, 1991b). Actin was cytochemically found to be most densely packed in the extreme apex of *Saprolegnia ferax*. Plastic changes in this actin cap and its disruption with cytochalasin E have led Jackson and Heath (1990) to propose a new theoretical model for apical growth complementing the debated ones previously proposed by Bartnicki-Garcia and Lippman (1972 in **I**) and Wessels *et al.* (1986 in **I**, 1988).

Exocytosis and apical growth may also be related as suggested by the restriction of glycoamylase secretion at the tips of growing hyphae (Wösten *et al.*, 1991). Temporal and spatial dynamic events intervening at appressorium formation in rusts are implied in the cessation of polarized tip growth and swelling of the hyphal apex (Kwon and Hoch, 1991).

Intracellular electric potential recordings in *N. crassa* hyphae have revealed a transfer of energy from proximal to apical cells (Potapova *et al.*, 1988). The galvanotropic response of growing hyphae was either mono- or bidirectional (longer hyphae) in an electric field (Gruler and Gow, 1990). Diffusion might be the mechanism

of bidirectional translocation of nutrients in mold hyphae (Olsson and Jennings, 1991). In the hyphal bundles of rhizomorphs of ectomycorrhizal basidiomycetes, translocation of solutes appears to be strongly polarized along concentration gradients towards sinks of various compounds (Cairney, 1992).

d) *Protonema (mosses)*

Chloronemal tip growth in *Funaria hygrometrica* is regulated by tip-localised H^+ secretion. From pH microelectrode measurements, Bittisnich and Williamson (1989) conclude that the "acid growth" hypothesis is applicable to tip growth in this moss.

e) *Prothallia (primary fern stage)*

Blue-light induces a mophological transition from a tip-growing filament to a planar prothallus (see Burgess, 1985 in **I**). Racusen *et al.* (1988) have shown that there is a rapid dissipation of both the longitudinally aligned electrical field and the tip-localized asymmetries in external cation distribution in blue-light, suggesting that "loss of electrical polarity in this tip growing cell may be an initial step in the chain of events which govern changes in cell shape".

f) *Pollen tubes*

Organelles in the subtip region of lily pollen tubes act as a sink for the calcium entering at the tip. The cytoplasmic gradient of calcium thus created, measured by microinjection of the indo-1 anion into pollen tubes by iontophoresis, is correlated with their growth (Rathore *et al.*, 1991). A distinct elevation of free intracellular calcium ion concentration has been measured at the extreme tip of actively growing *Lilium* pollen tubes by fluorescence ratiometric imaging (Miller *et al.*, 1992). Elevated levels of *membrane-bound* calcium had also been measured in lily pollen tube tip (Reiss and Nobiling, 1986) and the high $[Ca^{2+}]$ found with fura-2 at the same level is probably responsible for vesicle fusion at the tip (Obermeyer and Weisenseel, 1991).

g) *Root hairs*

The root hair membrane potential is depolarized by *Rhizobium meliloti* Nod factors (Ehrhardt *et al.*, 1992).

h) *Insect bristles*

Cell elongation determines the orientation of the axis of planar cell polarity which expresses itself as scales, hairs or bristles indicating thereby a supracellular tangential or "planar" tissue polarity (Nübler-Jung *et al.*, 1987), i.e. polarity in the plane of the cell sheet (Nübler-Jung and Mardini, 1990; Nübler-Jung and Eschbach, 1992, also see VII.C.6.b). Tissue polarity can be a manifestation of a gradient of cell adhesiveness

(Nardi and Kafatos, 1976), a gradient of a diffusible morphogen (Lawrence, 1966, 1970 in **I**; Stumpf, 1966), or direct cytoskeletal-plasma membrane connections between cells (Tucker, 1981, see also **I**). In this respect, the *Drosophila* tissue polarity gene *frizzled* (*fz*) is required to "coordinate the cytoskeletons of pupal epidermal cells so that a parallel array of cuticular hairs and bristles is produced" (Adler *et al.*, 1990).

In *Drosophila*, the genes *Notch* and *scabrous* participate to the specification of position-dependent cell fate of ommatidial founder cells and the formation of epidermal bristles in the adult epidermis (Hafen and Basler, 1991).

The interactions between the segment polarity genes have been analysed in order to unravel the different ways in which they contribute to epidermal patterning. The expression patterns of some of the segment polarity genes such as *wingless* and *engrailed* are spatially restricted (Hidalgo, 1991). There are similarities between these genes and those responsible for the dorso-ventral axiation (see VIII.B.2).

i) *Animal neurites*

The neuronal cytoskeleton has been recently reviewed (Burgoyne, 1991). The axonal cytoskeleton had been considered as a static complex travelling down the axon (Lasek and Black, 1988 in **II**), a view challenged by others (ref. in Okabe and Hirokawa, 1990). Recent results of analyses of the turnover of fluorescently labelled tubulin and actin in the axon of cultured neurons favor that view that these cytoskeletal filaments are dynamic structures that continue to assemble along the length of the axon (Okabe and Hirokawa, 1990).

The binding of microtubules-associated proteins (tau, etc.) to the microtubules might contribute to their stability in mature neurites. Consequently, tau antisense oligonucleotides can inhibit neurite polarity (Caceres and Kosik, 1990 in **II**).

Microtubule sliding has been reported to mediate axon elongation and tubulin transport (Cleveland and Hoffman, 1991). This sliding and slow axonal transport are powered by a common motor and by a molecule with the same polarity as microtubules such as dynein. However, Bloom's review (1992) points out to the new predictions emerging from the kinesin/dynein model for bidirectional transport (see IV.E.4). Otherwise, new evidence (Kuznetsov *et al.*, 1992, see IV.E.4) supports the hypothesis that both actin-based (actomyosin-like mechanism) and microtubule-based motility systems are associated with each other to produce and regulate the movement of organelles in axoplam.

The functional polarity of nerve cells requires targeting of microtubular components which are asymmetrically distributed. There is a known difference in the polarity of the microtubules in the axon and the dendrites (Black and Bass, 1989). In the axon, the polar orientation of microtubules is uniform with assembly ends (+) pointing away from the cell body whereas in dendrites microtubules are mixed with both (+) and (-) ends pointing away from the cell body. It remains to know "how the neuron selectively directs the specific mRNA into its dendrites and axon and whether microtubules are involved in this process" (Ginzburg, 1991). Additional factors, independent of microtubule polarity orientation, might also contribute to the targeting mechanism. It was indeed shown that

the synaptic terminals are deprived of neurofilaments or microtubules (Lasek and Hoffman, 1976) and that their disaggregation would be due either to a higher calcium ion concentration (Lasek and Hoffman, 1976) or the action of a calcium-activated protease (Roots, 1983). A possible role of proteases secreted by the growth cones in neurite polar advancement has again been suggested (Pittman *et al.*, 1989 in Bixby and Harris, 1991). During axonal elongation, microtubule translocation is the principal means of tubulin transport (Reinsch *et al.*, 1991). However, the mechanism of such transport and the location of polymer assembly are presently unknown.

A review on functions of nerve growth cones at the tips of elongating axons and dendrites (Cypher and Letourneau, 1992) cites two monographs about them (Burgoyne, 1991; Letourneau *et al.*, 1991). Growth cones can distinguish one group of neurons from another. This guidance ability is called selective fasciculation mediated by fasciclin II, a member of the immunoglobulin superfamily. This molecular model confirms in *Drosophila* "the existence of functional labels on specific axon pathways in the developing nervous system" (Grenningloh *et al.*, 1991).

The "neuroblast identity gene" *prospero* (*pros*) regulates other neuronal precursor genes and encodes a nuclear protein which is essential for the axonal outgrowth and pathfinding of central and peripheral neurons (Vaessin *et al.*, 1991).

The synergistic action of three known cell surface molecules (L1, N-cadherin and integrin) and additional unidentified components concour to the polarized elongation of peripheral axons (Bixby *et al.*, 1989; Rathjen, 1991). In the regulation of axonal growth by gradients of chemotropic molecules intervene many proteins among which cell adhesion molecules ("neurite outgrowth promoting molecules", see Bixby and Harris, 1991). Spatial gradients of axon guiding molecules would provide positional and directional cues for retinal ganglion cell axons growing within the optic tectum (Baier and Bonhoeffer, 1992).

The orienting role of electric fields and the galvanotropic response of nerve cells as well as the problem of their "normal regeneration which utilizes the electric field as part of the signaling process to attract neurites to the regenerating region" have been reviewed by Nuccitelli (1988). Of interest was the observation by McCaig (1986) that the electric field can influence neurite morphology by creating a cell asymmetry only after neurite outgrowth.

By combining neurophysiological principles with silicon engineering, Mahowald and Douglas (1991) have produced an analogue integrated circuit with the functional characteristics of real nerve cells (see also IV.B.2.e). For their "neuromime" they have built a silicon microchip circuit that mimics the electrical polarized behaviour of real nerve cells (Andreou, 1991).

B. Bipolar growth

a) *Bacterial elongation*

A FtsZ protein has been found to self-assemble as a ring structure at the future division site of the cell of *Escherichia coli* (Bi and Lutkenhaus, 1991). As tentative

model to explain the central ring's localization (homobipolarity), it has been proposed that the cell poles at old sites can compete with the medial cell site (Begg and Donachie, 1977 in **I**, and 1985).

C. Multipolar

c) *Desmidial algae (multiradiate pattern)*

As already mentioned (Brower and Giddings, 1980, also in **I**) the lobes of *Micrasterias* exhibit galvanotropism toward the cathode while those growing toward the anode tend to be shorter (Brower and McIntosh, 1980).