Zeitschrift: Archives des sciences et compte rendu des séances de la Société
Herausgeber: Société de Physique et d'Histoire Naturelle de Genève
Band: 38 (1985)
Heft: 3

Artikel: Another model of cell polarity : the outgrowing fungal spore
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DOI: https://doi.org/10.5169/seals-740490

Arch. Sc. Genève	Vol. 38	Fasc. 3	pp. 419-426	1985

ANOTHER MODEL OF CELL POLARITY: THE OUTGROWING FUNGAL SPORE

BY

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KEYWORDS: Polarity, fungal spore, *Neurospora,* apical acidification

ABSTRACT

Polar patterns of a few fungal types are presented comparatively to polarity of hydra. Growth at germination of *Allomyces* species is bipolar as budding in the polyp. According to our simpler model of monopolar germination from conidia of *Neurospora crassa,* axial polarity would be initiated by vectorial extrusion of H^+ ions from a few mitochondria positioned toward the thereby elected plasmalemma site of outgrowth. The ensuing localized acidification would initiate a train of ionic exchanges and vectorial transports (acidic proteins, vesicles) leading to germ tube elongation.

RÉSUMÉ

Les types de polarité de quelques champignons ont été présentés par comparaison avec celle de l'Hydre. La croissance à la germination des espèces d'*Allomyces* est bipolaire comme celle du polype. Selon notre modèle plus simple de germination monopolaire de la conidie de *Neurospora crassa,* la polarité axiale serait induite par extrusion vectorielle d'ions H^+ à partir de quelques mitochondries positionnées contre le site plasmalemmique ainsi élu pour l'émergence. L'acidification localisée en découlant mettrait en marche une chaîne d'échanges ioniques et de transports vectoriels (protéines acides, vésicules) conduisant à l'élongation du tube germinatif.

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The polarity of hydra, well-known since the celebrated Trembley's regeneration experiments, has its prototypic counterparts in lower eucaryotic organisms such as the Algae (Nuccitelli, 1983) and the Fungi (Harold, 1982; Turian, 1983a). These last present a whole array of polar patterns (Figure 1) spanning from the monopolar budding of a simple yeast such as *Saccharomyces cerevisiae* to monopolar outgrowth of a germ tube from conidia of *Neurospora crassa* or, more primitively, a rhizoid from the subspherical thallus of the water mold *Blastocladiella emersonii.* The bipolar pattern is reached with germinating zoospores of the more evolved water molds of the genus *Allomyces:* first a rhizoid emerges from a pole apparently predetermined by the flagellum site on the zoospore (Turian, 1962) and, second (around 1 h later) at the opposite pole, the hyphal tube elongating at 180° of the rhizoid and soon regularly dichotomizing into a tufted mycelium (Figure 2).

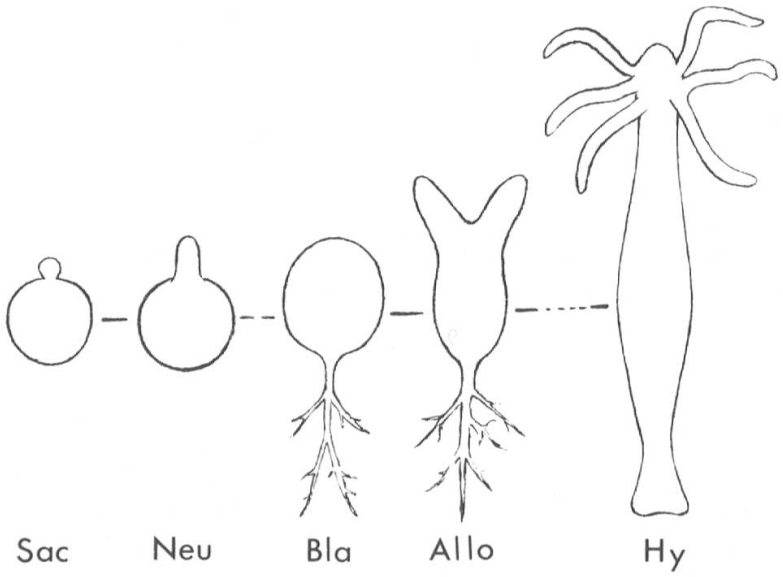

FIG. 1. — Comparative polar pattern from germinating fungal spores to growing Hydra (Hy): unipolar budding in *Saccharomyces cerevisiae* (Sac), unipolar outgrowing conidium of *Neurospora crassa* (Neu), unipolar rhizoid outgrowth from *Blastocladiella emersonii* (Bla) zoospore, bipolar axial rhizoid—hypha outgrowths from *Allomyces arbuscula* (Allo) zoospore.
Size scale of the longest fungus (Allomyces ≅ 25 μ) compared to the polyp equals 1 to 50.

The site of polar budding on mother yeast cells has been variously considered to be predetermined by vectorial "bombardment" of its wall by cytoplasmic granules (Falcone and Nickerson, 1958) or endomembranar vesicles (Moor, 1967), frontal preorientation of the spindle polar body preparing first mitotic division (Byers and Goetsch, 1975) or positioning of actin dots below the emergence site (Kilmartin and Adams, 1984). Our contribution has been the detection, with fluorescent probes, of an early acidification in the outgrowing yeast bud (Turian, 1981).

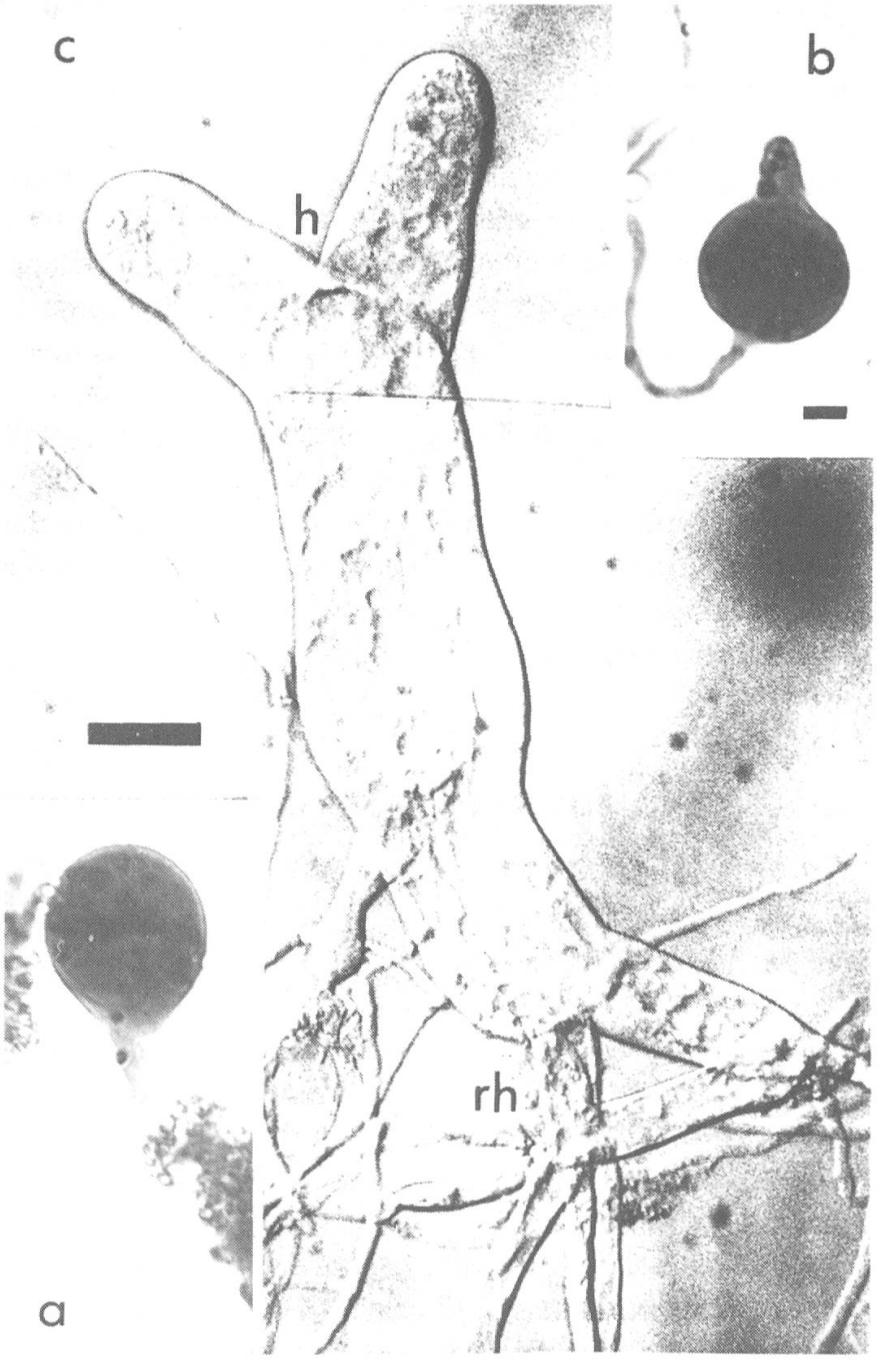

FIG. 2. — A bipolarly axiated germling of *Allomyces arbuscula* photographed on a Nomarski equipped Olympus microscope.
Note the young hyphae (h) dichotomously expanding from the apical pole of the main mycelial "trunk", itself issued by enlargement of the initially germinated zoospore having first (a) outgrown the basal rhizoidal system (rh). Bar = 10 μ.

Our main effort to understand the establishment of a polar axis at fungal spore germination has been centered on *Neurospora* conidia. As in all other spores of Ascomycetes and Fungi imperfecti, the germination process includes three main stages: (1) the "dry" stage covering the dormancy (ascospores) or semi-dormancy (conidia) period; (2) the "wet" stage initiating "swelling" or isotropic growth of the spore; (3) the outgrowth or emergence stage expressing the polarity of growth which continues with apical elongation of the hyphal tube.

During the early isometric growth phase, the organelles are homogeneously distributed in the cytoplasm. Internal partition of these constituents intervenes only later at the stage involving clustering of mitochondria at a pole opposite to the vacuoles-enriched one (Turian, 1985). Such polar clustering of mitochondria appears to be a prerequisite to the second, more decisive event, the temporary contact of a few of such mitochondria with a localized portion of the plasma membrane (Turian and Geissler, 1984), electing by that presumably electrically-depolarizing event, the prospective site of germ tube outgrowth. This site is differentially acidified (Turian, 1981, 1983a, 1983b) and known to be progressively filled with vesicles fusing with the apically expanding plasma membrane (Gooday, 1983) and insuring by their provision of wall material the elongation rate of the hyphal tip. The differential acidification of the prospective site of germ tube outgrowth has been ascribed to the vectorial ejection of protons from frontal mitochondria toward a cytosolic sink (Turian, 1980).

PROTON SINK THEORY FOR THE OUTGROWTH OF THE GERM TUBE

As proton sink, we had suggested a process of ionic exchange such as H^+/K^+ at the plasmalemma site facing these mitochondria (Turian, 1983c). K^+ ions are effectively required for germ tube outgrowth (Turian *et al.,* 1984) but their requirement appears to be related to an early exchange with protons extruded from the initially relatively acidic semi-dormant conidia to insure the relative alkalinisation of the cytoplasm compatible with DNA synthesis and first mitosis at germination. Such initially ejected protons could then be reintruded in symport with glucose and/or aminoacids into the expanding germ tube while peripherally increasing the pH of the surrounding medium as recently measured with a vibrating electrode (Kropf *et al.,* 1984). Such protons reintrusion would reinforce the apical acidity initiated by the vectorial ejection of protons from the frontal mitochondria (Figure 3). Part of the apical acid charge might concentrate into the vesicles migrating to the hyphal tip, some at least being suspected to function therefore as chromaffin vesicles in a process which should involve the functioning of membrane H^+-ATPase consuming mainly glycolytically-produced ATP since frontal mitochondria are suspected to be partially uncoupled (Turian and Michéa-Hamzehpour, 1983). Apical vesicles could therefore act as sink

for protons on their vectorial move to the germ pole. As in germ tubes of the alga *Fucus* (Quatrano *et al.,* 1978), arising vesicles are probably negatively-charged by their load of wall polysaccharidic precursors. Therefore, they could attract, on their way from the endoplasmic reticulum to the tip, the protons ejected from the frontal mitochondria (Figure 3) as well as being attracted to the electropositively-charged outgrowing tube.

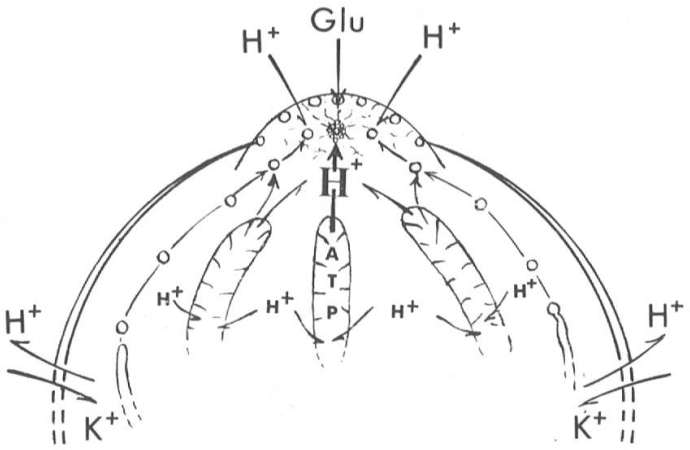

FIG. 3. — Diagrammatic sketch of the partially hypothetic, sequential polar process of germ tube outgrowth from a *Neurospora* conidium. Initial endogenous vectorial flush of protons (H^+) from the frontal mitochondria progressively retreating from the electrically depolarized site of the outgrowing germ tube. Apical proton sink self-amplified by migration of negatively-charged vesicles attracts exogenous protons cotransported with glucose (Glu) according to Harold's report. Such protons should be available following their outward exchange with K^+ ions previously intruded to deacidify the cytoplasm of the conidium from its isometric "swelling" stage on. Contrarily to the uncoupling state of the mitochondrial frontal zones, basal areas could reintrude protons to replenish the ATP pool by chemiosmotic oxidative phosphorylation. ATP is requested for further energized extrusion of H^+ into the apical dome. ATPase-mediated protons sequestration into exocytotic vesicles would provide the necessary apical sink for protons vectorially conducted by actin "cables" of the microfibrillar network.

The cytosol progressively sorted out at the tip should be gelified (cytogel) by the ensuing local acidification as known in the ectoplasm of myxamoebae (Condeelis and Taylor, 1977). We had suspected (Turian, 1979) that this apical gelification might be related to changes in the organisation of the meshwork of actin microfibrils which have since been cytochemically detected in budding yeasts (Adams and Pringle, 1984) and fungi (Hoch and Staples, 1983; Ton-That and Turian, 1983). The extension of the gelified microfibrillar network would progressively push-back the frontal mitochondria, leaving at the ultimate tip a restrictive or exclusion zone of cytogel accepting only the several types of vesicles among which the micro-type aggregated into the "Spitzenkörper". Microfilaments of actin could themselves be electrically polarized in the axis of the apical acid gradient and, in their interaction with vesicles, might contribute to their electrophoretic transport toward the outgrowing tip.

In the sequential morphogenesis of the fungal germ tube we could thus consider that, complementary to the initial asymmetric turgor pressure, clustering the mitochondria to the prospective outgrowing pole (Turian, 1985), the interaction microfibrils-vesicles along the pH gradient would provide the electro-mechanical driving force for the polarized traffic of the wall-building material toward the germ tube transformed into a continuously elongating hyphal tip.

Polarized growth is, however, not only sustained by apically-driven traffic of wall and plasmalemma vesicles but, more quantitatively, by a continuous supply at that hyphal tip site of cytosolic and enzymatic proteins. The driving force of such a transport depends upon the accumulation of H^+ ions in the tip of the germ tube which provides there a positive charge by contrast with the basal portion of the germinative spore; the accompanying transcellular ion currents should generate an intracellular, apico-basal voltage gradient in the germling. In the exclusive zone of the tip, above

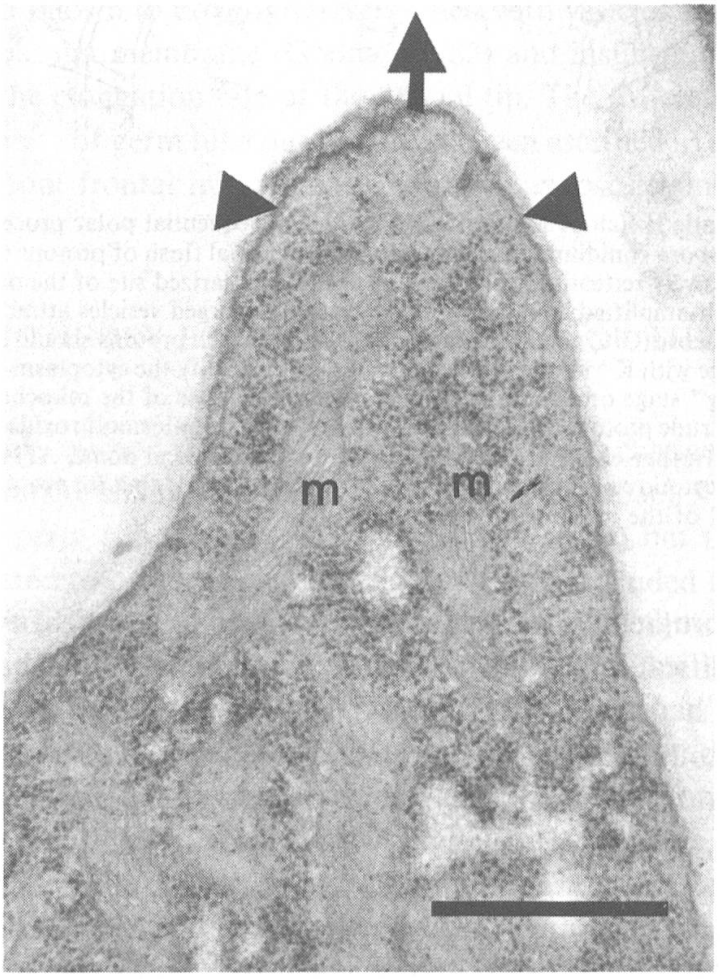

FIG. 4. — Longitudinal section of a germ tube outgrowing (arrow) from a conidium of *Neurospora crassa*. The ribosomes are excluded from the cytosolic ultimate tip observed to be the most acidic by fluorescence quenching of acridine orange.
Mitochondria (m) in subapical position. Fixation in glutaraldehyde-osmic acid. Bar = 1 μm.

the frontal mitochondria, that voltage gradient could be involved in the polarized apical transport of proteins released from the free ribosomes immobilized on the subtip front of the hyphal apex (Figure 4).

The reason of such exclusion phenomenon could be found in the known fact that ribosomes having their protein component rich in basic aminoacids and conferring them a high pI, they should have a higher amount of positive charges in the acidic apical environment (fluorometric probing of pH $\sim$ 5.5, see Turian 1983b) and therefore would tend to be repelled from that most acidic—anodic—ultimate tip. Oppositely, the mainly acid, electro-negative proteins and enzymes (low pI such as invertase, calmodulin, actomyosin, etc.) presumed to be produced on the free ribosomes of that subtip region would be vectorially discharged toward the tip by a process akin to "bioelectrofocusing", thereby insuring the constant sorting out of fresh apical cytosol required for the continuous hyphal elongation. In such acidified conditions, that cytosol should be gelified (Turian, 1979) into some type of "ectoplasmic cap" imprisoning among its actomyosinic, fibrillar network the apical vesicles sequestering H^+ ions (proton sink, Turian, 1983c) and wall precursors.

Fundamentally it then appears that it is the maintenance of the apical endo- and exogenous proton provision which would insure, by further electrically-controlled asymmetrical release of newly formed proteins, the continuous polarized hyphal growth.

We are grateful to the organizers of the symposium for the opportunity to compare our views on fungal polarity with those on hydra in a "témoignage d'admiration pour notre grand ancêtre intellectuel genevois, Abraham Trembley". We thank Dr. M. Ojha and Dr. T. C. Ton-That for the photomicrographs of *Allomyces* and *Neurospora* respectively.

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