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Polarized acidification at germ tube outgrowth from fungal spores (*Morchella* ascospores, *Neurospora* conidia)

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Abstract

The cytosol sorted out from the mitochondria-containing cytoplasm in a polar fashion at the onset of germ tube emergence from conidia of *Neurospora* or from ascospores of *Morchella* turns pH-indicator dyes such as bromocresol green or alizarin S to a yellow acid tinge indicating a pH <5. Such localized acidification is confirmed by the quenching of the fluorescence of acridine orange in the emerging buds and tips of germ tubes outgrowing from *Neurospora* conidia.

Three types of structural changes forming part of the same process can be recognized during germination of fungal spores: spore swelling, germ tube emergence and germ tube elongation (Allen 1965, Gottlieb 1978). However, the swelling is not a simple consequence of the initial «reawakening» hydration of the spore but very soon involves synthesis of new proteins, and should more adequately be called isometric (isotropic) growth (Bartnicki-Garcia 1981). Emergence or outgrowth of germ tube(s) would then correspond to the first stage of anisometric or anisotropic growth which is of fundamental importance for the normal developmental sequence leading to new hyphal growth of the fungus. Unfortunately, the mechanism of its inception has as yet remained elusive even though its initial event could be ultrastructurally described as the appearance of a vesicle-rich polar zone (Hemmes and Hohl 1971, Grove and Bracker 1978). This zone which becomes the so-called exclusion zone (amitochondrial) at the tips of elongating hyphae has previously been cytochemically characterized by both its reducing capacity (Turian 1978) and its relatively low pH (Turian 1979a). Such apical acidity detected with colorimetric indicators has also been found in the germ tubes outgrowing from *Neurospora* conidia and suggested to be of physiological significance (Turian 1980).

It was therefore of interest to confirm the localized acidification not only in extending the semi-vital color reactions to wider germ tubes such as those outgrown from *Morchella* ascospores, but in applying a more refined technique, that of the fluorescent probe acridine orange (Nuccitelli and Deamer 1982), to the germ tubes of *Neurospora*.

Material and Methods

Ascospores of *Morchella conica* Pers. (var. *costata* Vent.) were scratched from commercial, dry carpophores and dispersed into an aqueous, 1% solution of malt (Berthet 1964). After 8-9 h at 20°C, drops of the suspension of ascospores in early germination were flattened below coverslips; pH-indicators (10^{-3} w/v in distilled water) were added laterally to obtain gradients of concentration.

Conidia of *Neurospora crassa* were obtained from 10-15 d old slant cultures on solid Vogel's medium (1956). They were dispersed in Vogel's medium ($\sim 10^6$/ml) and drops of the suspension were allowed to germinate on slides in humid chambers at 20°C. After 3 h incubation, the early germinating conidia were spread below coverslips and stained with the pH-indicators gradientially added as above.

As pH-indicator dyes we used bromocresol green, bromocresol purple, and alizarin yellow S (corresponding reagent for Ca^{2+} ions) while chlorophenol red served for additional checkings. The internal pH was estimated by comparison of the tinges in spores chosen in nonoverstaining zones of the color gradient with the color scales of Langeron (1934) and Drawert (1968). Color photomicrographs were made on a Wild-M20 microscope with Agfa color (400 Asa) film for *Neurospora* conidia and Fujicolor F-II film for the ascospores of *Morchella*.

The fluorescent pH-probe acridine orange (Merck products) was freshly dissolved in distilled water (10^{-4} M). One drop of it was added to one drop of conidial suspension and the flattened preparation immediately screened for color photomicrographs with Ektachrome film on a Leitz Dialux 20 epifluorescence microscope.

Results

With germinating ascospores of *Morchella* we obtained our clearest results with alizarin yellow S but could not avoid reddish precipitates in the medium (glycoproteinic exsudates?). Optimal focusing on a young germ tube just emerging in a clear yellow color from a pole of an ascospore (Plate 1,1) revealed both its acidity (pH <5) and low Ca^{2+} content by contrast with the red color of the less acidic but calcium-richer cytoplasm of the ascosporal body.

Plate I.

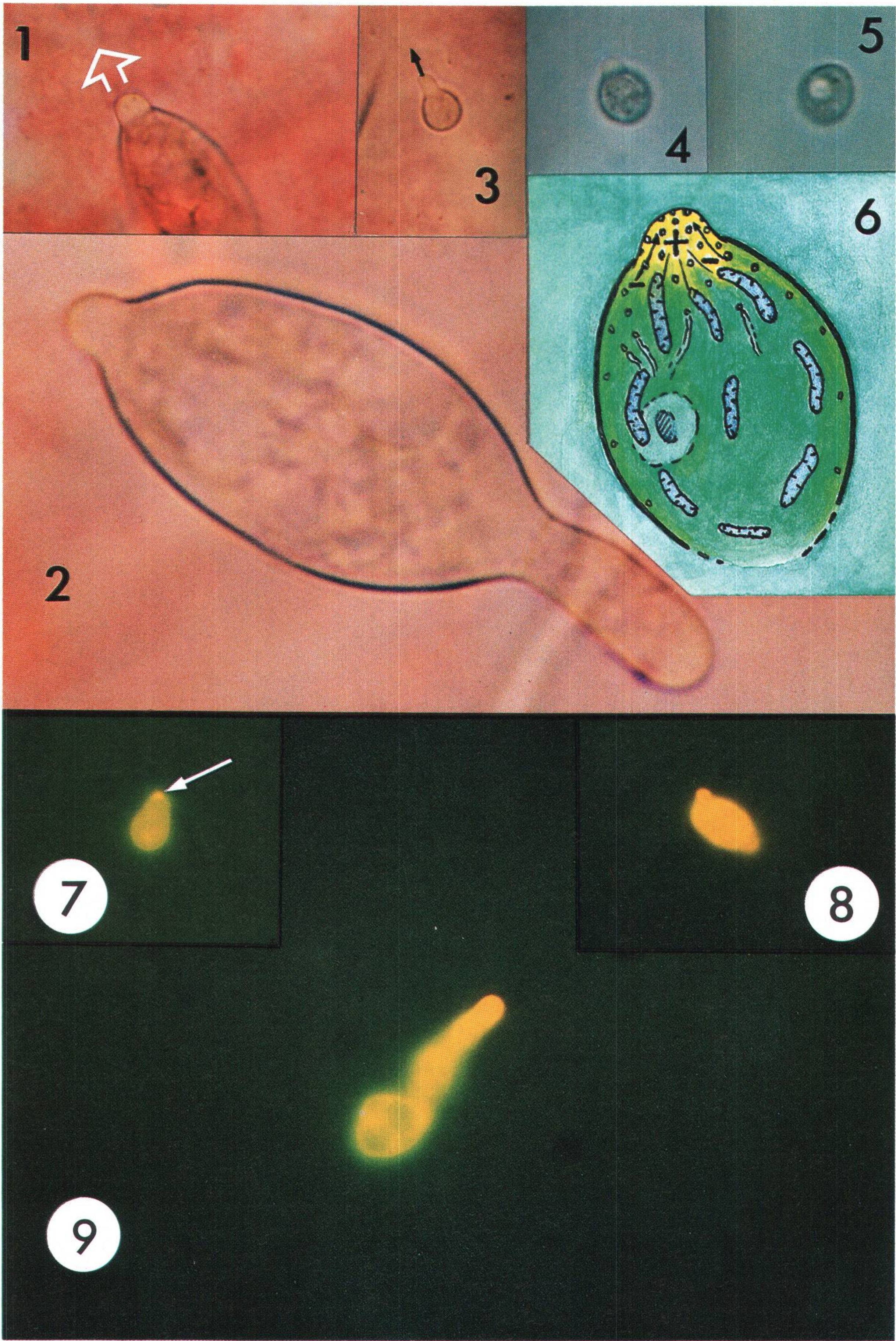
Semi-vital stainings of germinating spores with pH-indicators (1-6).

A. *Morchella conica* ascospores:
1. In alizarin yellow S, emergent (arrow) yellow acid bud, ×1200.
2. Bipolar germination in bromocresol purple, × 3000.

B. *Neurospora crassa* conidia:
3. In alizarin yellow S, yellow germ tube (arrow), × 800.
4. In bromocresol green, outgrowing acidic bud (side view), × 1000.
5. Idem, frontal view, × 1000.
6. Ultrastructural-topological diagram of differential pHs in a germinating conidium as indicated by bromocresol green switched to yellow (pH <4.5) in the outgrowing bud, bluish-green (pH >5) in the general cytosol and blue (pH >6) in the mitochondria. Protons vectorially dissipated into the emerging tip (+) attract the vesicles fusing their negatively (–) charged content into the extending tip wall.

pH-fluorescence probes (7-9).

7. Fluorescence of acridine orange quenched at the presumptive germination site (arrow), × 1500.
8. Idem in the germ bud, × 1500.
9. Idem at the tip of the germ tube, × 1500.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Bromocresol purple having a low permeability rate was slower to reveal the contrast between the yellowish germ tubes and the purplish staining of the ascosporal body, especially noticeable at the periplasmic level (Plate 1,2). With bromocresol green, the ascospores appeared dark green, with a bluish hue in the peripheral layers and a greenish-yellow tinge in the outgrowing buds.

On germinating conidia of *Neurospora,* we had to add a drop of a nearly saturated solution of alizarin yellow S in distilled water to observe a sharp contrast between the yellow outgrowing germ tube and the reddish content of the conidial body (Plate 1,3). Here also, cloudy reddish precipitates formed in the dispersion fluid, probably in contact with conidial exsudates.

In bromocresol green, germ buds outgrowing from conidia stained yellow (pH ~ 4.5), in sharp contrast to the general bluish-green staining (pH > 5) of the conidial body (Plate 1,4) in which, at high focusing, a few blue mitochondrial granules could be visualized. The yellow tinge of the buds observed laterally was maintained in frontal views (Plate 1,5), a fact indicating that the switch from green to yellow was not due to an optical effect of restriction of the light absorption path in the narrow bud structures. In bromocresol purple or in chlorophenol red, the outgrowing buds appeared yellowish, in contrast to the general red hue of the conidial bodies. However, permeation of these stains through the conidial walls was generally poor and the increased concentration required, rather than improving the internal, semi-vital staining, often directly led to a lethal acidification of the whole conidial contents.

With acridine orange, the yellowish-green fluorescence conferred to the cytoplasm of the conidial bodies was repeatedly observed to be quenched to an orange tinge in the germinating structures. Such orange fluorescence due to local acidification signalled the initial cytosolic bumps (Plate 1,7), filled the young buds (Plate 1,8) and «capped» the elongating germ tubes (Plate 1,9).

Discussion

Local cytoplasmic acidification parallels germ tube outgrowth from two types of fungal spores, as shown by the yellow switch at their level of all pH-indicator dyes tested. This has been further evidenced by the use of the fluorescent pH-probe acridine orange which, as a weak base, accumulates in acidified cell compartments; there, its yellowish-green, monomer fluorescence is more or less intensively quenched to a polymeric fluorescence appearing red in some highly acidified animal cells (Plates in Nuccitelli and Deamer 1982) or orange as in our more moderately acidified fungal structures. With another fluorescent pH-probe, 4-methyl-esculetin, we had already observed quenching of the fluorescence but to darkness in yeast buds and hyphal tips of *Allomyces,* indicative of an internal pH value of not more than 5 in such emergent structures (Turian 1981); such estimation is in good agreement with the average pH value of 4.5 estimated now from the yellow tinge observed with indicator-dyes in outgrowing buds of *Morchella* and *Neurospora.* Even admitting that this low value of pH could be upgraded to 5.0 by taking into account the possible «color shift» of the indicators due to salts and protein effects (Drawert 1968), such pH remains clearly lower than those measured in a few vegetative fungal structures. They span from the still relatively acidic values of 5.8-6.0 in yeast cells as measured by nuclear magnetic resonance technique (Gillies *et al.* 1981) to 7.1 in *Neurospora* hyphae as determined with microelectrodes (Sanders and Slayman 1980).

With our simple microscopic method of the pH-indicator dyes, we have observed that the general cytoplasm, mainly the cytosol, of the sporal bodies stained bluish-green with bromocresol green (reddish-purple with bromocresol purple) corresponding to an average pH value of 5.5 while its mitochondrial granules appearing clear blue have a pH of at least 6. When compared to the values obtained with the sophisticated techniques, ours are therefore under-estimated by about 1 pH unit. However, they do not detract from the fact that the cytosol of the germ buds, stained yellow with the same reagents, is the most acid. We cannot therefore escape the main conclusion that the polarized inception of fungal germ tubes is paralleled by the onset of a downhill pH gradient spanning from the perimitochondrial cytosol of the sporal bodies to the cytosol sorted out into the expanding germ buds. A diagrammatic color representation illustrates such downhill gradiential distribution of the internal pHs in an early germinating conidium of *Neurospora* (Plate 1,6).

The first event in the inception of the pH gradient has been hypothesized as a vectorial dissipation of protons from randomly back-positioned mitochondria (Turian 1979b). Such vectorial protonation of a cytosolic zone would locally depolarize the plasmalemma, while determining the presumptive site of germ tube emergence.

Continuity of the outgrowth process would require polar sorting out of acidified cytosol from the mitochondria-containing cytoplasm self-entrained by ionic exchanges (H^+-K^+ especially) through the apically depolarized plasmalemma functioning as a proton sink for the vectorially deprotonated frontal mitochondria (Turian 1980).

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Résumé

Le cytosol séparé polairement du cytoplasme peuplé de mitochondries lors de l'initiation de l'émergence du tube germinatif à partir des conidies de *Neurospora* ou des ascospores de *Morchella* fait virer les colorants-indicateurs de pH tels que le vert de bromocrésol ou l'alizarine S à leur teinte acide jaune (pH <5). Une telle acidification localisée est confirmée par le changement de fluorescence de l'orange d'acridine dans les bourgeons en émergence et les extrémités de tubes germinatifs sortant des conidies de *Neurospora*.

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