

# Further clues for the involvement of mitochondria in the initiation of germ tube outgrowth from fungal conidia (*Neurospora* model)

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## Further clues for the involvement of mitochondria in the initiation of germ tube outgrowth from fungal conidia (*Neurospora* model)

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### Abstract

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Close proximity of mitochondria with the prospective site of germ tube outgrowth signalled by a slight protuberance on “swollen” conidia has repeatedly been observed in *Neurospora crassa*.

In 1976, Burnett (p. 230) credited us with the first report about “an increase in mitochondrial numbers” at fungal spore germination. Our work was concerned with the sequential outgrowth of the rhizoid and the hyphal tube at opposite poles of zygotes of *Allomyces* (Turian 1958). Since then a few other papers, also cited by Burnett, have confirmed this topocytological situation, most prominent among them the work of Rosen et al. (1974) dealing with germination of *Trichoderma* conidia.

The functional significance of a local clustering of mitochondria was still unclear. Recently, a clue was obtained from our work on mitochondria which apparently are involved in the oriented delivery of protons into the developing germ tube tip. The latter functions as an acidic sink attracting apical vesicles (Turian 1980, 1983 a). The initial event whereby the choice of the prospective site of outgrowth was determined remained, however, elusive. We postulated the presence of a few mitochondria either some distance from the site of germ tube initiation (Turian 1979 a) or perhaps initially even in contact with the plasma membrane at that site. The lowering of the pH would then cause the cytoplasm to gel and push the mitochondria to a more posterior position (Turian 1983 b). Encouraging such a contact hypothesis was an ultrathin section of a swollen conidium of *Neurospora crassa*, obtained in 1974 in collaboration with Mrs N. Oulevey and clearly showing partition of the cell into a predominantly vacuolized pole opposite a mitochondrially enriched pole. This arrangement somehow appeared to pre-determine the position of the germ tube initial. The later was already visible as a slight protuberance of the membrane-wall system and contained a cluster of mitochondria, two of which appeared to be in very close proximity to the plasma membrane.

The second line of evidence was obtained using enforced isometric growth at 46 °C followed by shift-down at 25 °C which induces polarized growth (Ton-That & Turian

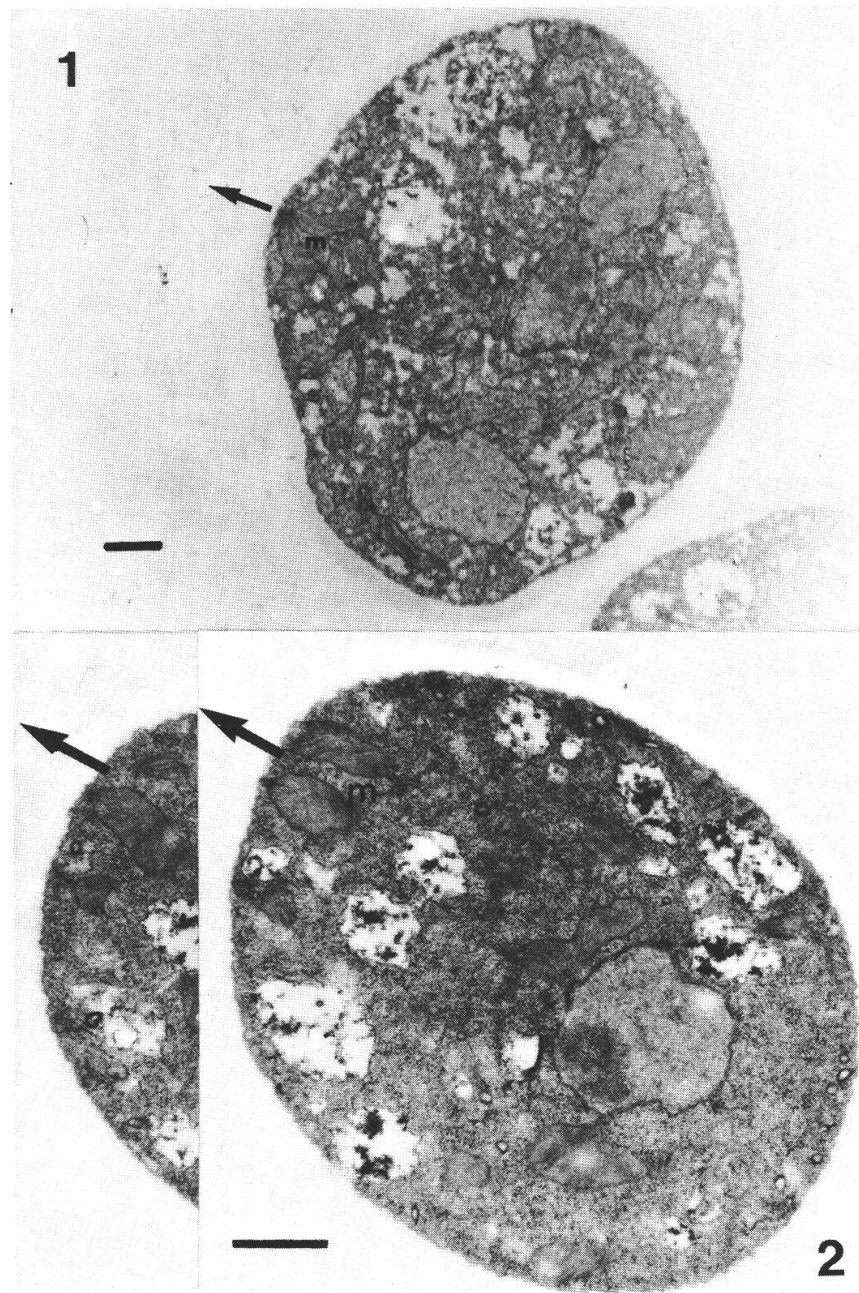


Fig. 1. Conidium of *Neurospora crassa* at its pregermination "swollen" stage after 2 h of incubation at 25 °C in Vogel's liquid medium containing a stimulatory concentration ( $10^{-5}$  M) of 2,4-dinitrophenol. Two mitochondria (m) in the initial bump (arrow indicates the direction of expansion) pointing to the membrane site of germ tube outgrowth. Fixation in glutaraldehyde (2.5%) –  $\text{OsO}_4$  (2%), staining and poststaining in uranyl acetate (2% aqueous) and lead citrate. Grids observed in a Zeiss EM 10. Bar = 1  $\mu\text{m}$ .

Fig. 2. Close association of polarly-oriented mitochondria (m) with the prospective plasmalemma site of germ tube outgrowth signalled by a slight peripheral bump (arrow of directional expansion). Note the outward orientation of the cristae in these mitochondria appearing to contact the cytoplasmic membrane through patches of electron-dense material. The mitochondrion at the lower cell periphery parallels the plasmic membrane without, however, contacting it. Insert, next section of the same conidium. Fixation, idem to Fig. 1. Bar = 1  $\mu\text{m}$ .

1978). One of these enlarged, overswollen conidia transferred for 4 h at 25 °C displayed internal features also suggesting its cytoplasmic polarization along a longitudinal axis. The latter ended with two axially oriented mitochondria almost in contact with the membrane of the prospective site of outgrowth (Ton-That 1979, Fig. 51).

This striking, local interaction between the mitochondrial and the cytoplasmic membranes has since been observed in a section of a pregerminating conidium of *N. crassa* incubated in the presence of  $10^{-5}$  M 2,4-dinitrophenol, an uncoupling agent known to dissipate proton gradients, while stimulating both latent mit-ATPase and conidial germination (Turian & Michéa-Hamzehpour 1983). In the early cytoplasmic protuberance two mitochondria are axially oriented toward the outgrowing cytoplasmic membrane which they appear to contact through an intermediary patch of electron-dense material (Fig. 1).

In our most recent effort to follow up this promising new interpretation of morphogenic determination by intracellular positioning of a cluster of mitochondria, we have applied the technique of serial ultrathin sectioning to swollen conidia at their pre-emergence stage (hydrated for 1 h before fixation). In one series we confirmed the presence of mitochondria which were oriented parallel to the prospective axis of germination (Fig. 2). In this figure two of these mitochondria are shown in two consecutive sections in close contact with the cytoplasmic membrane. The contact site is marked by an electron-dense patch of material.

The perpendicular positioning of mitochondria at a precise site of the conidial cytoplasmic membrane suggests the possibility of its local depolarization by means of a proton flow from the tips of these mitochondria. The latter might be uncoupled by some interaction (visualized as the patchy material?) between mitochondria and cytoplasmic membrane. The localized acidification of the cytosol might then initiate the gelified exclusion zone (Turian 1979 b) which progressively excludes the mitochondria from the elongating hyphal tip while attracting numerous cytoplasmic apical vesicles.

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## Résumé

Une proximité étroite de mitochondries avec le site présomptif d'émergence du tube germinatif, repéré par une légère protubérance sur les conidies « gonflées », a été observée à plusieurs reprises chez *Neurospora crassa*.

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