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Decreasing pH-gradient toward the apex of germinating pollen tubes

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We have recently described the tip of the elongating hypha as a proton sink of gelated hyaloplasm. This could provide electrochemical motive power for the acropetal transport of vesicles containing wall precursors thus ensuring polarized growth (Turian, 1978-80).

The purpose of this study is to extend our findings from fungi to elongating pollen grain tubes. Like hyphae, actively growing pollen tubes show tip zonation, organelles being excluded from the clear apical zone of the tube (Heslop-Harrison, 1979). Such a topological situation makes them an especially favourable material for cytochemical-physical studies concerning polarity phenomena.

Material and methods

Pollen grains were harvested from mature stamens from commercial strains of either the daffodil, *Narcissus pseudonarcissus* or the amaryllis, *Hippeastrum vittatum*. For germination, they were dispersed in a tap-water solution of sucrose (2 %) and boric acid (0.02 %) in small flat glass containers maintained at 20 °C in a wet chamber. After 2-4 h, drops of the suspensions of partially germinated grains (from their early emergence stage to that of germ tubes 2-3 times the width of grains) were pipetted on slides. Drops of the pH indicators (10^{-3} w/v in distilled water) were then pipetted on one side of the coverslip and a gradient was formed by blotting on the opposite side. Observations and photomicrographs were made only on germ tubes stained in vital, or at the most, subvital, conditions which were characterized by the active cytoplasmic streaming in a germ tube still moderately elongating (Fig. 1). Such favourable materials were easily contrasted with the few lethally overstained pollen tubes recognized by their clotted, uniformly acidified cytoplasm. The pH-indicators used were bromocresol purple (BCP) or bromocresol green (BCG), occasionally bromothymol blue and Congo red, all estimated for their colour pH-scale according to Langeron (1934) and Drawert (1968). A Wild-M20 microscope and Ilford Pan F and Fujicolor F-II400 films were used.

The fluorescent probe 4-methylesculetin (6,7 dihydroxy-4-methyl-coumarin, Senn Chemicals) was added as a drop of a saturated solution in distilled water and the photomicrographs made after intracellular penetration, on an Ortho-lux Leitz microscope equipped with UV excitation system (350 nm) and filters cutting off fluorescent light emitted between 420-450 nm according to Gerson and Burton (1977).

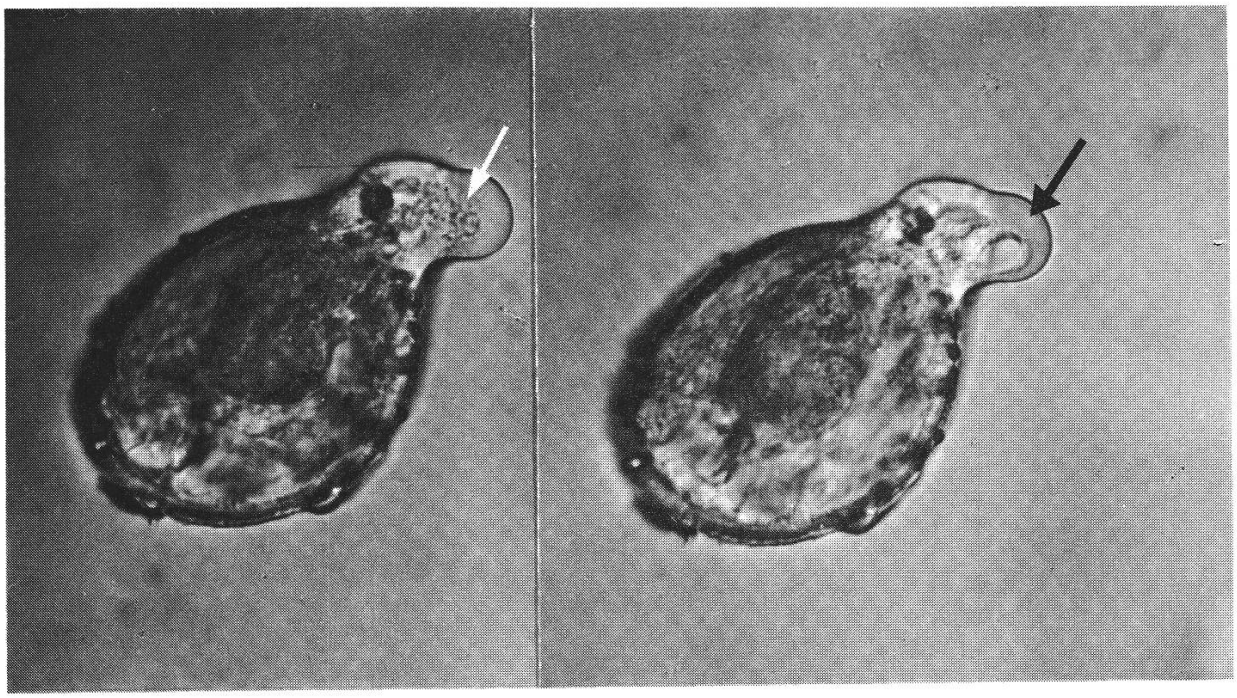

Fig. 1
Emergent tube of daffodil pollen stained with bromocresol green, photomicrographed at 5 min intervals through a blue filter: greenish-blue, granular zone of mitochondria (white arrow) below moving front of yellowish hyaloplasm (black arrow). × 500.

Results and Discussion

In the middle zone of its concentration gradient, bromocresol purple stained the cytoplasm purplish and the mitochondrial rods denser purple in the elongating germ tubes of daffodil pollen (4 h); this was in sharp contrast to the straw-coloured staining of the domed tip. That this switch of BCP to yellow in the apex was not due to any optical reflection could be checked by serial focusing (Plate I,a).

In the more concentrated range of BCP staining, emerging tubes of amaryllis showed even clear jellow staining of the «capping» tip zone (Plate I, b); this sharp contrast of the yellow tips with the purplish subapical zone containing dark purple mitochondrial rods was also seen in elongating germ tubes of amaryllis (Plate I,c).

From the above colour switches to yellow of BCP in the tips of emerging and elongating germ tubes, the pH of such vesicles-containing hyaloplasm was rated at the average value of 5.0. In contrast, the purplish hue of the hyaloplasm embedding mitochondria in the subapical zone is indicative of a pH close to 6.0. Dark purple mitochondria would have a pH ranging around 6.5 as also suggested by the greenish hue observed in a few sublethal stainings with the classical bromothymol blue.

Confirmation of the acropetally decreasing gradient of pH first observed with BCP was obtained with bromocresol green, a semivital pH indicator first proposed by Yamaha (1935) for the generally acidic plant tissues. Our gradiential diffusion of BCG showed many emerging apices of both daffodil and amaryllis pollen stained yellowish-green in the domed zone (pH not more than 5.0 but above 4.5 as indicated by the lack of a switch to blue of Congo red) with a green subapical hyaloplasm (pH ~ 5.5) embedding the blue (sharp focusing) mitochondrial rods (pH at least 6.0–6.5) of the collar zone (Plate I,d).

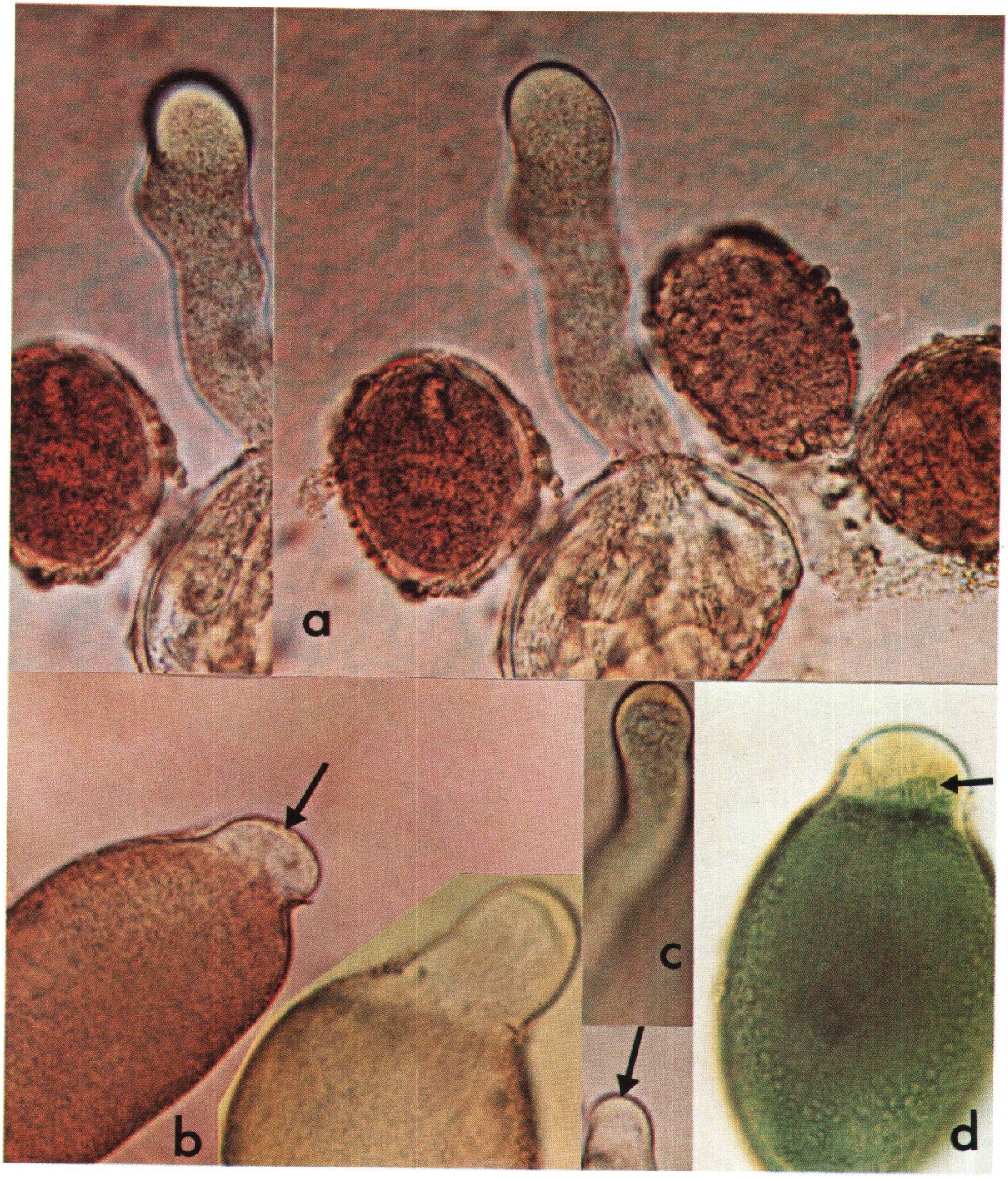

Plate I.
Stainings of germinating pollen tubes with colorimetric pH-indicators.
Bromocresol purple:
a. Two focuses on the domed tips of a germ tube of *Narcissus pseudonarcissus*.
b.c. Emerging (b) and elongating (c) tubes of *Hippeastrum vittatum*. Arrows on yellow «cappings». × 400.
Bromocresol green:
d. Emerging tube of *Hippeastrum vittatum*. Arrow on mitochondrial zone. × 400.

The fluorescent probe 4-methylesculetin is an easily penetrating reagent that fluoresces from around pH 5.5 (Gerson and Burton, 1977). When germinating pollen grains of daffodil were bathed in its saturated solution (a few small fluorescent crystals eventually remaining in suspension) for 1/2-1 h, they exhibited fluorescence in the cytoplasmic body of the grains and only up to the few mitochondrial rods visible in the collar of the emerging tube; the whole domed tip was extinct (Plate II,a). Plunging pollen grains with elongating germ tubes into the solution of 4-methylesculetin excited fluorescence over practically their entire length, except for a cap remaining extinct (Plate II,b). Such general lack of fluorescence in the tips is indicative of a hyaloplasmic apical pH lower than 5.5 and therefore again close to an average value of 5.0. By contrast, the vivid greenish-grey fluorescence of the subapical mitochondria indicates a pH of at least 6.0. The permeation of mitochondrial membranes by 4-methylesculetin was confirmed by the lack of fluorescence in no-probe controls.

The pH gradient that we detected in the germ tubes of pollen is rather similar to that found in hyphal tubes, namely with a subapical pH averaging 6.0 in its hyaloplasm and around 6.5 in its mitochondria, while decreasing to a low pH 5.0 in the hyaloplasmic apex. The relatively low pH $\sim$ 5.0 detected in the emerging and elongating tips should favour a gel rather than a sol condition of their hyaloplasm, by analogy with what has been described in amoebae pseudopods (Condeelis and Taylor, 1977). It also implies a low Ca^{2+} level in such ultimate tips, an imbalance between calcium ions and acidity being known to lead to apical bursting (Dow and Rubery, 1975). In consequence, the Ca^{2+} gradient detected in growing pollen tubes by Jaffe *et al.* (1975) and Reiss and Herth (1978) should be attenuated in their tips as suggested by some results of these last authors and visualized in hyphal tips by alizarine S red switch to yellow (Turian, 1979a). Relevant to such Ca^{2+} restriction in the tips is the fact that exogenous calcium ions blocked germination of *Hordeum* pollen (Heslop-Harrison, 1979 Plate 18) as could be expected from an overwhelming of the low Ca^{2+} level and the low pH, both required for the gelation of the ultimate tips.

Acidic cytogel in the apices of outgrowing pollen tubes could, by analogy with the situation proposed for fungal hyphal tips (Turian, 1979b), also be considered as a proton sink initiated by the vectorial dissipation of H^+ ions from initially asymmetrically positioned mitochondria. The electrochemical gradient thus formed should then attract negatively charged vesicles containing wall-precursors and enzymes to the positively charged apex. That the apices of pollen tubes are positively charged has already been deduced from experiments showing selective entrance at this site of K^+ ions in exchance of H^+ ions extruded from inside the tube (Weisenseel *et al.*, 1979). Recent experiments with effectors of mitochondrial $H^+(Mg^{2+})$ ATPase suggest that these protons originate from the mitochondria (Turian, 1980), in conformity with our vectorial dissipation model (Turian, 1979b).

The cytoplasmic vesicles are known to originate behind the subapical front of the mitochondria, namely from the Golgi elements (Franke *et al.*, 1972); their acropetal movement has variously been ascribed to cytoplasmic streaming implicating microfilament sliding (Franke *et al.*, 1972) or to self-electrophoresis (Jaffe and Nuccitelli, 1977). Our present experiments suggest that the decreasing gradient of pH from $\sim$ 6 in the perimitochondrial cytosol (zone of reprotonization by active respiration and phosphorylative coupling) to a value of $\sim$ 5 in the amitochondrial, apical cytogel could well be sufficient to provide electrochemical driving force for the acropetal migration of these vesicles.

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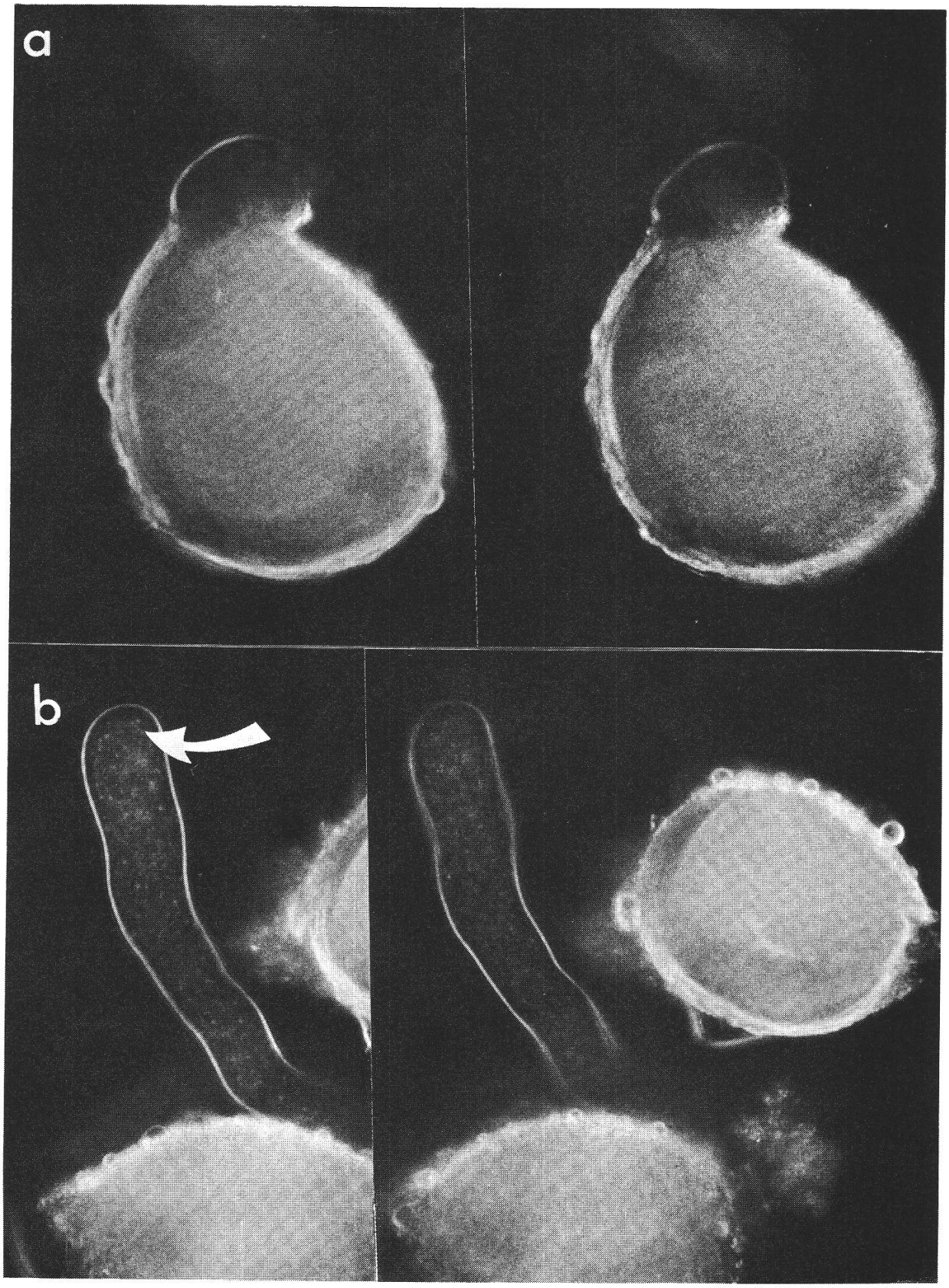

Plate II.
Extinction of the greenish-grey fluorescence of the pH-probe 4-methylesculetin in the domed tip of emerging (a) and ultimate tip of elongating (b) pollen tubes of *Narcissus pseudonarcissus*. White arrow on subapical mitochondrial zone. × 700.

Summary

In the domed tips of emerging and elongating pollen germ tubes of daffodil and amaryllis, the colorimetric pH-reagents bromocresol purple and bromocresol green switch to their acid yellow tinge indicating an average pH of 5 of the hyaloplasm.

Such an acropetally decreasing pH gradient in the pollen tubes is confirmed by the apical extinction of the greenish-grey fluorescence of the pH-probe 4-methylesculetin, especially vivid in the mitochondria-rich, subapical zone (pH > 6).

Résumé

Les réactifs colorimétriques de pH, pourpre de bromocrésol et vert de bromocrésol, virent à leur teinte acide jaune indicatrice d'un pH moyen de 5 du hyaloplasme dans les extrémités arrondies des tubes polliniques en émergence et en élongation de jonquille et d'amaryllis.

Un tel gradient acropète décroissant de pH dans les tubes polliniques est confirmé par l'extinction apicale de la fluorescence gris verdâtre du réactif de pH 4-méthylaesculine spécialement vive dans la zone subapicale riche en mitochondries (pH > 6).

Literature

Condeelis J.S. and D.L. Taylor (1977). The contractile basis of amoeboid movement. V. The control of gelation, solation and contraction in extracts form *Dictyostelium discoideum*. J. Cell Biol. *74:* 901-947.

Dow J.M. and P.H. Rubery (1975). Hyphal tip bursting in *Mucor rouxii:* antagonistic effects of calcium ions and acid. J. Gen. Microbiol. *91:* 425-428.

Drawert H. (1968). Vitalfärbung und Vitalfluorochromierung pflanzlicher Zellen und Gewebe. Protoplasmatologia. Band II, D, 3, 749 pp. Springer-Verlag, Wien.

Franke W.W., W. Herth, W. J. Van Der Woude and D.J. Morré (1972). Tubular and filamentous structures in pollen tubes: possible involvement as guide elements in protoplasmic streaming and vectorial migration of secretory vesicles. Planta *105:* 317-341.

Gerson D.F. and A.C. Burton (1977). The relation of cycling of intracellular pH to mitosis in the acellular slime mould *Physarum polycephalum*. J. Cell. Physiol. *91:* 297-305.

Heslop-Harrison J. (1979). Aspects of the structure, cytochemistry and germination of the pollen of rye *(Secale cereale* L.). Ann. Bot. *44* (Suppl. 1): 1-47.

Jaffe L.F. and R. Nuccitelli (1977). Electrical controls of development. Ann. Rev. Biophys. Bioeng. *6:* 445-476.

Jaffe L.F., M.H. Weisenseel and F. Jaffe (1975). Calcium accumulation within the growing tips of pollen tubes. J. Cell Biol. *67:* 488-492.

Langeron M. (1934). Précis de Microscopie, 1205 pp. Masson et Cie, Paris.

Reiss H.D. and W. Herth (1978). Visualization of the Ca^{2+} gradient in growing pollen tubes of *Lilium longiflorum* with chlorotetracycline fluorescence. Protoplasma *97:* 373-377.

Turian G. (1978). The «Spitzenkörper», centre of the reducing power in the growing hyphal apices of two septomycetous fungi. Experientia *34:* 1277-1279.

Turian, G. (1979a). Cytochemical gradients and mitochondrial exclusion in the apices of vegetative hyphae. Experientia *35:* 1164-1166.

Turian G. (1979b). Polarity of elongation growth generated and sustained by anisotropic distribution of the protons ejected from mitochondria into the cytosol of hyphal apices *(Neurospora* model)? Arch. Sc. Genève *32:* 251-254.
Turian G. (1980). Germination of conidia *(Neurospora, Trichoderma):* evidence of an electrogenic sink of protons mitochondrially-extruded into emerging tubes. Bull. Soc. Bot. Suisse *90* (3/4): 203-212.
Weisenseel M.H., A. Dorn and L.F. Jaffe (1979). Natural H^+ currents traverse growing roots and hairs of barley *(Hordeum vulgare* L.). Plant Physiol. *64:* 512-518.
Yamaha G. (1935). Über die pH-Schwankung in der sich teilenden Pollenmutterzelle einiger Pflanzen. Cytologia *6:* 523-526.

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