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BIOVOLTAIC POLARIZATION OF TIP GROWING CELLS: THE FUNGAL SPORE MODEL

[POLARISATION BIOVOLTAÏQUE DES CELLULES À CROISSANCE APICALE (SPORES FONGIQUES COMME MODÈLES)]

BY

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ABSTRACT

Bioenergized charge separation between mitochondrial matrix ($^-$) and cytosolic proton sink ($^+$) is considered as a biovoltaic polarizing effect initiator of acid tip growth examplified by fungal spore germination.

More than 50 years ago, Lund (1928) suggested that the sources of bioelectric phenomena might be related to the redox reactions of respiration. This idea was developed by Lundegärdh (1939) who suggested that when the cytochrome system, of which the terminal member is inhibited by cyanide, was reduced by accepting an electron into the iron atom of the porphyrin, a proton was released simultaneously to the medium. He suggested that this separation of charge might bring about further movement of other ions. After that, Conway and Brady (1948) published the first suggestion that protons released at the cytochrome system might be the source of the hydrogen ions of the HCl secretion by the gastric mucosa. Therefore, the quantity of HCl secreted would be related to the O_2 uptake of respiration. The fact that the ratio of HCl/O_2 was rather high (up to 12/1) led two research workers (Davies and Ogston, 1950) to propose that some additional H atoms and hence, ions and electrons might also be derived from the phosphate carriers. Such a proposal was suggestive of an interdependent relationship between phosphorylation and the

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separation of charge at the electron carrier and chemical mechanisms implicating energized intermediaries were first proposed to explain this oxidative phosphorylation (Slater, 1966).

An alternative mechanism for the formation of ATP, the so-called chemiosmotic hypothesis, for the formation of ATP has however been put forward from 1961 by Peter Mitchell. Further developing Robertson's idea (1960) that a charge separation occurs in mitochondria and chloroplasts and is responsible for the formation of ATP form ADP + P_i, Mitchell suggested that the synthesis of ATP might be due to a reversal of the reaction of an hydrolytic ATPase associated with the mitochondrial internal membrane. The reversal would occur when the ATPase, oriented in a particular way in the membrane, is subjected to the separated H and OH ions. Phosphorylation would then be due to the pH shift produced across the membrane thereby reversing to a synthase effect the mitochondrial ATPase activity (Mitchell, 1966).

Evidence from a different source suggested by 1960 another possible charge separation of physiological importance, that occurring in chloroplasts in light. Such light-inducing effect of charge separation has been further confirmed by Jagendorf (see Miller, 1979) on isolated chloroplasts in which it is now understood as a process akin to the photovoltaic effect.

This mechanism of light-induced charge separation is exploited in man-made solar cells or silicon photovoltaic cells in which an electric potential develops across the p (positive) — n (negative) charges junction. Energization of the process of charge separation (positive "holes" versus electrons) is insured by the protons emitted under steady illumination of the crystalline silicon. The effectiveness of the photovoltaic cells rests on their inherent asymmetry and the ability of the junction (doped with trace elements "impurities") to keep the positive and negative charges separate.

In the absence of photons, energy for charge separation could be provided by a proton gradient artificially created by acidification (ΔpH) across the thylacoid membrane of chloroplasts and leading to total dark synthesis of ATP (Jagendorf and Uribe, 1966). In mitochondria, the energy for building-up proton gradient can be provided by either premade mitochondrial ATP (ATPase proton pump) or by the oxidative processes of respiration (redox pump) ejecting out couples of protons through the internal membrane of the mitochondria.

In the Mitchell's model or chemiosmotic theory, the charge separation process is usually considered at the short-range level of mitochondrial membranes, the protons ejected being sometimes viewed as remaining between internal and external membranes before their "vectorial" re-intrusion. However, as stated by Skulachev (1981), "the scheme of the proton cycle does not show how far H^+ ions penetrate the water phase after crossing the membrane. They could enter the bulk water phase or, under certain conditions, $\Delta\mu H$ generators and consumers might operate

so fast and could be localized so close to each other, that protons do not leave the membrane/water interface." In their critical, "in vitro" experiments of 1965, Mitchell and Moyle have shown that when a buffered suspension of mitochondria with a respiratory substrate such as succinate and initially deprived of oxygen was aerated, hydrogen ions were rapidly ejected into the liquid medium. In an "in vivo" situation, such protons would be extruded from the mitochondria into their surrounding cytosol, this last being acidified by comparison with the relatively alkalinized organelles. The ΔpH between the mitochondrial matrix and the cytosol has been evaluted by the P_{31} NMR technique and found to oscillate between 0.3-0.8 in vegetative yeast cells (Shulman et *al.*, 1979). Similarly, bacteria which can be considered as free mitochondria acidify their nutritive medium by a proton extrusion expected to be isotropic around their cells.

Now, if we take the position to equate to such liquid media the cytosol of cells and consider that the cytosol surrounding the mitochondria is topologically equivalent to the liquid growth medium of bacteria, we can therefore apply the "in vitro" results on the acidification of the medium to the *intra*cellular inter-relationship between mitochondria and their surrounding cytosol, the hyaloplasm of the cytologists. Charge separation might then occur between mitochondrial matrix and cytosol separated by the mitochondrial membrane(s) which should provide the necessary tough barrier to maintain the difference in charge that has built up, as well as to store that energy for a brief period. We must however take into account the fact that there is a regulatory device to control the rate of re-entry of protons. As Nicholls (1982) states "The factor which actually controls the rate of respiration is the extent of disequilibrium between the redox potential spans across the proton-translocating regions of the respiratory chain and $\Delta\mu_H{}^+$ (the electrochemical potential difference, in mV, for protons between two bulk phases separated by a membrane, sometimes called protonmotive force). In State 4 (when ADP is exhausted), respiration is automatically regulated so that the rate of proton extrusion by the respiratory chain precisely balances the rate of proton leak back across the membrane. If proton extrusion were momentarily to exceed the rate of re-entry, $\Delta\mu_H{}^+$ would decrease, the disequilibrium between the respiratory chain and $\Delta\mu_H{}^+$ would decrease, and the proton current would in turn decrease, restoring the steady-state." However, we can imagine intracellular situations in which protons extruded in excess would be somehow prevented from being re-intruded into their mitochondrial source. Such a diversion could be provided by a proton sink which would create an asymmetrical cytotopological situation. This idea led us to favour the hypothesis (Turian, 1979a) of an anisotropic re-intrusion into the mitochondrial matrix of the isotropically extruded protons rather than that of a possible anisotropic extrusion of protons. In such a view, a certain proportion of the extruded protons would be vectorially dissipated toward a cytosolic sink created by the aleatorily retreated positioning of a group of mitochondria from the periplasmic zone in swollen, pregerminated fungal

spores. Such vectorial dissipation of protons from mitochondria toward a cytosolic sink (Turian, 1980) would thus be directionally inverse to the "uphill" ATP generating-gradient of protons.

To establish and maintain a cytoplasmic gradient with ions as mobile as H^+ across the mitochondrial membrane(s) must require the expenditure of a great amount of energy; that could be met first by the electrogenic proton-translocating ATPase found to be activated at the germ tube outgrowth (Turian and Michéa-Hamzehpour, 1983). The initial protonmotive potential is therefore built up by hydrolysis of endogenous ATP (presynthesized in the sporal mitochondria) and the net direction of such protonmotive potential, out of the mitochondrial matrix, is expected to be that of the direction of ATP hydrolysis. Such ATPase pump is expected to be further relayed by the redox pump activated at the elongation of the germ tube (Schmit and Brody, 1976). As both of these protonmotive energizations are biochemically produced, we propose the term *biovoltaic* to designate such effect of self-entrained (source and sink) and maintained charge separation by endogenous, life processes.

The cytoplasmic gradient of protons would not only be positioned by reference to the mitochondrial membrane(s) but to the initially symmetric topology of the whole cell, in our example, the swollen fungal spore. The initial flux of non-reintruded (dissipated) protons would therefore contribute to the first asymmetrical sorting out of acidified cytosol from the mitochondria-rich zone and the positioning of the first, peripheral crescent of acidified cytosol enriched with vesicles (as revealed by pH indicators, Turian, 1980, 1983a) is the first functional sign of primary axiation inside the relatively isometric spore before its polarized actualisation as an out-growing germ tube.

Our model cells have been germinating spores of fungi without predictable site of germ tube outgrowth namely conidia of the *Monilia* type *(Neurospora crassa, Sclerotinia fructigena)* and of *Trichoderma harzianum* (Turian, 1980) as well as ascospores of *Morchella conica* (Turian, 1983a). The answer to their unpredictable polarity of germination appears to lie in an aleatory grouping of mitochondria occurring after the "swelling" or isometric growth phase of germination favoring a vectorial dissipation of protons (see above). In spores provided with a pre-built germ pore (ascospores of *Chaetomium*, basidiospores of *Coprinus*, etc.), we are led to think that the hydrolytic products of the plugging poral material somehow orient the initial grouping of mitochondria. However, in both cases, we are left with the problem of unravelling the nature of the proton sink necessarily postulated to understand the diversion of the protons from their mitochondrial source and therefore the maintenance of the charge separation, the so-called biovoltaic effect, between the negatively charged mitochondrial matrix and the most positively charged apical cytosol (Figure 1). As proton sinks, we have envisaged two possibilities which could be complementary:

1. The ionic exchanges intervening at the plasmalemma site facing the frontal mitochondria across the cytosolic and vesicle-rich exclusion zone of the typical growing hypha. Local, tip membrane depolarization could be expected from such cationic exchanges primarily implicating H^+/K^+ and $2H^+/Ca^{++}$ (see Harold, 1977; Slayman, 1981). With Ca ions, the acidification of the apical cytosol could be amplified by exchanges with protons (see Meech and Thomas,

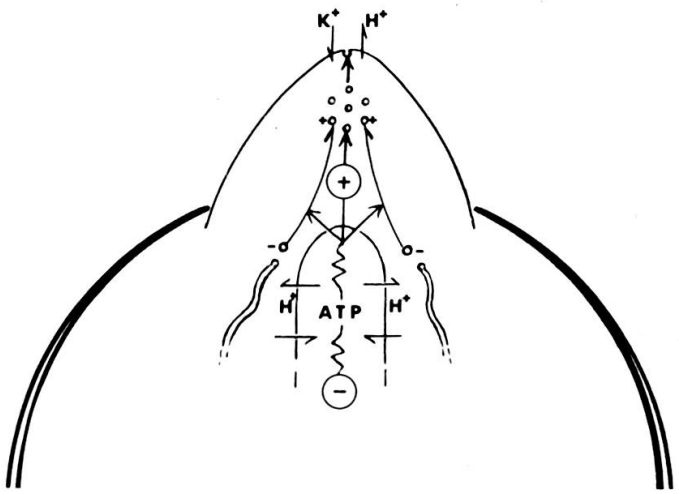

FIGURE 1. — Polarized outgrowth of the germ tube from a fungal spore model (*Monilia* type).

Protons (H^+) extruded from frontal mitochondria by hydrolytic splitting of ATP are vectorially dissipated toward the cytosolic sink expanding as the exclusion zone at the tip of the elongating hyphal tube.

As apical proton sink could be considered: (a) negatively-charged microvesicles budded from endomembranar elements and attracted to the positively-charged, acidic "Spitzenkörper" before further acropetal fusion with the apically expanding plasmic membrane, and (b) ionic exchanges ($K^+ \rightleftharpoons H^+$; $Ca^{2+} \rightleftharpoons 2H^+$?) at this last level.

1980) across the membranes of frontal mitochondria appearing to sequester Ca^{2+} (Turian, 1979b). Such a role of ionic exchanges is also apparent in our recent observation of the contingent role of K^+ for germ tube emergence from conidia of *Neurospora crassa* (Loeffel, Michéa-Hamzehpour and Turian, in preparation). However, such a view of ionic exchanges as peripheral proton sink may be limited in its generalization when we consider the possibility of germ tube outgrowth from "dry" conidia in a relatively wet atmosphere, as examplified by conidia of Erysiphales. On "dry" slides in petri dishes simply humidified by a moderately wetted blotting-paper, conidia of *Microsphaera alphitoides* or of *Erysiphe communis* outgrew aerotropically their germ tubes which showed acid, yellowish tips when bathed in alizarin yellow S or in bromocresol green (unpublished observations).

Moreover, it is well known that aerial hyphae can elongate on long distances to colonize a distant substrate. This also invites us to be cautious with the apical ionic exchanges only, even though acropetal migrations of the necessary ions through the water imbibed hyphal walls cannot be excluded, and to consider the possibility of an additional (rather than fully alternative) endogenous source of apical proton sink.

2. Some type of the numerous vesicles known to migrate from the endomembranar system to the hyphal tip. This is suggested by the known fact that chromaffin vesicles in hepatic cells can sequester protons from the acidified cytosol and concour there to the homeostatic pH regulation of the cytoplasm (Pace et al. 1982). At tip growth, protons first vectorially dissipated from the frontally positioned mitochondria into the cytosol of the presumptive hyphal tip can be entrapped on their way into large or small negatively charged vesicles (with aminoglycosylated content, etc.) themselves attracted by the positively charged zone surrounding the fronts of the grouped mitochondria. Originally, we were struck by the vivid yellow tinge produced by the "Spitzenkörper" — an apical aggregate of microvesicles among others — in germinating conidia of *Neurospora crassa* bathed in our pH indicators (Turian, 1979b). We have therefore some experimental reasons to think that these microvesicles are attracted by the general positive charge of the apical cytosol and captate protons on their way from the endoplasmic reticulum (Najim and Turian, 1979) to their fusion site with the apical plasmic membrane of the elongating septomyceteous hyphae (see Figure 1). Such intramicrovesicular enrichment in H ions, presumably associated with the presence of the coenzyme NAD ($NAD^- + 2H^+$), might have relevance to the high reducing power cytochemically detected in the "Spitzenkörper" (Turian, 1978). Additional protons dissipated into the cytosol might contribute by Gibbs-Donnan equilibrium (De Duve et *al.*, 1978) to the acidification of the lysosome-type of apical vesicles cytochemically demonstrated to have acid phosphatase activity (Dargent and Denisse, 1976, in Grove, 1978).

It should be emphasized that it was no hazard that we could propose this biovoltaic model of cell growth polarization by using germinating fungal spores. From its emergence, the hyphal tip can be considered as an exclusion zone, deprived of mitochondria on a 5 μ length; the first of these organelles are oriented parallely to the longitudinal hyphal axis and therefore offer a dead-end front of proton extrusion into a "vacuum zone" (cytosol plus vesicles) without possible interaction with neighbouring mitochondria to re-intrude surrounding protons vehiculated by cytosolic streaming currents. Such exclusion zone shown cytochemically to be acidic (Turian, 1981, 1983a, b) has been discussed above as the sink for vectorially dissipated protons (see Figure 1) while frontal mitochondria keep the negative charges as

partners in the apex-determining or polarizing bioseparation of charges, the so-called biovoltaic effect.

As a conclusion, the preexistent energy (ATP) and the newly produced one (by redox reaction) available in a germinating fungal spore provoke a charge separation across the membrane(s) of mitochondria between positive charges (protons) in the cytosol and negative charges in their matrix. Lasting charge separation and therefore full biovoltaic effect is only achieved when a positioned grouping of mitochondria directs vectorial dissipation of excess protons momentarily ejected (at inception of germ tube outgrowth) toward a cytosolic-vesicular sink, thereby creating the biovoltaic polarization or bioelectrical axiation of the developing hyphal tube. The biovoltaic field thus created may be the device to move self-electrophoretically the vesicles toward the expanding membrane and wall required by hyphal tip growth. Fungal hyphae — and other tip growing cells (Sievers and Schnepf, 1981), such as pollen tubes (Turian, 1981) and possibly nerve growth cones — appear therefore as ideal structural models of spatial segregation at opposite ends of proton pump(s) and proton sink(s) ensuring by biovoltaic effect the setting up of a transcellular electrochemical gradient generative of growth polarity.

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