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# Transcellular ionic currents studied by intracellular potential recordings in *Neurospora crassa* hyphae

## Transfer of energy from proximal to apical cells

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Membrane potentials, input resistances, and electric coupling in the apical parts of *N.crassa* growing hyphae were recorded with the aid of intracellular microelectrodes. It was revealed that the apical cells were always depolarized by 10 to 30 mV as compared to the adjacent proximal cells. The septal pore maintained an electrical resistance of 4 to 6 MΩ. The calculated values of the endogenous electrical current passing through the septal pore varied between 0.5 and 1 nA. Electrical isolation of the apical cells resulted in their depolarization from 120–150 mV to 40–60 mV, characteristics of the membrane potential value of *N.crassa* adult hyphae with completely blocked electrogenic pumps. A simultaneous increase in the input resistance value from 15–20 MΩ to 40–80 MΩ was observed. The above data can be explained assuming that $H^+$-ATPase activity was greatly lowered in the apical cells. Thus in the intact hyphae with electrically coupled cells energy is transferred from the proximal hyphal compartments to the apical ones.

Intercellular junction; Ionic flux; Energy transfer; Plasma membrane $H^+$-ATPase; Hyphal growth; (*Neurospora crassa*)

## 1. INTRODUCTION

The possibility of energy distribution between adjacent cells was previously substantiated for various multicellular systems via ionic fluxes that pass through permeable cell-to-cell junctions [1,2]. Cells with available electrogenic pumps provide the adjacent cells with inactive primary transport systems with energy essential to support electrochemical potential gradients. Such an energetic cooperation is supposed to exist between hyphal cells of the fungus *N. crassa*, where a membrane potential heterogeneity was established between the electrically coupled cells [3,4].

In several studies of the past two decades, extracellular proton currents which provided for the polarized extension of the *N. crassa* hyphae were observed using a vibrating probe and pH sensitive microelectrodes [5,6]. To account for the above data it hsa been proposed that the apical cells of the hyphae were relatively deficient in $H^+$-ATPase [6,7].

The purpose of this study was to test the ability of hyphal apical cells to maintain their membrane potentials ($E_m$) at the expense of endogenous mechanisms of active transport and to estimate quantitatively the electric currents passing through permeable cell-to-cell junctions (septal pores) in the growing hyphal apex.

## 2. MATERIALS AND METHODS

The work has been performed with *N. crassa* wild strain R-2 [8]. The culture used was grown for 22–24 h in Petri dishes on cellophane discs covering the Vogel's agar medium with 2% sucrose. The cultural conditions, the methods of mycelium

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preparation and the methods of microelectrode measurements are described elsewhere [9,10].

In our experiments glass micropipettes were filled with 1 M potassium citrate (resistance of the microelectrode $\geqslant$50 M$\Omega$). The $E_m$ value, the input resistance ($R_{input}$) value and the coefficient of the electrical coupling between the cells ($K_c$) were obtained with the aid of two intracellular microelectrodes (fig.1).

Electrophysiological measurements were performed in a pH 6 solution, containing 1.2 mM $Na_2HPO_4$, 8.8 mM $NaH_2PO_4$, 8.8 mM NaCl, 10 mM KCl, 1 mM $CaCl_2$, 85 mM sucrose.

The attachment of the hyphal apex was realized by a glass microhook moving with the aid of a micromanipulator. Electrical isolation of the apical cell was obtained by means of a mechanical injury of the adjacent trunk cell with a glass microneedle.

## 3. RESULTS

In the apical cells of intact *N. crassa* hyphae the values of membrane potentials were 120–145 mV. Stable polarization of *N. crassa* hyphae was observed: $E_m$ of the growing apical cell was always 10–30 mV lower than $E_m$ of the proximal cells at a distance of 200–500 $\mu$m from the tip (table 1, fig.2). The internal voltage gradient in the *N. crassa* hyphae was previously obtained by Slayman and Slayman [12].

Electrical uncoupling at a distance of 150–300 $\mu$m from the tip always evoked drastic depolarization of the apical cell up to 40–60 mV (table 1) coupled with an increase in the $R_{input}$ value from 15–20 M$\Omega$ to 40–80 M$\Omega$. Electrical uncoupling of the apical parts of the hyphae contain-

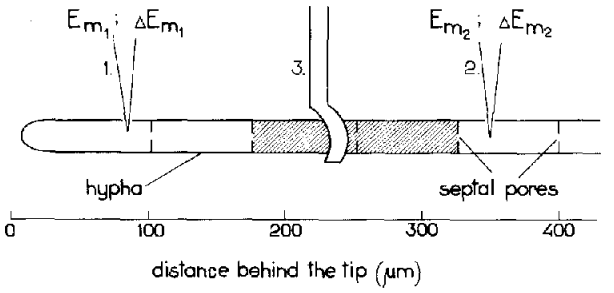


Fig.1. A scale diagram of the arrangement of microelectrodes in the apical zone of the *N. crassa* hyphae. Two microelectrodes (1 and 2) are inserted simultaneously into the two cells to measure the membrane potentials ($E_{m1}$ and $E_{m2}$) and their electrotonic changes ($\Delta E_{m1}$ and $\Delta E_{m2}$) after the added current pulses (1 nA, 100 ms) pass through a microelectrode penetrating cell 1. The ratio of the $E_m$ shift in the apical cell (in response to the current pulses) to the value of the current pulse is taken for the cell input resistance value ($R_{input}$). The ratio of the $E_m$ shift in cell 2 (in response to the current pulses passing through the cell 1) to the $E_m$ shift in cell 1 was calculated as a coefficient of the electrical coupling between the cells ($K_c$).

$$R_{input} = \frac{\Delta E_{m1}}{J}; \quad K_c = \frac{\Delta E_{m2}}{\Delta E_{m1}}$$

The shaded area shows the zone of mechanical injury. The septal pore was plugged as a result, and the apical cell became electrically isolated from the rest of the hypha. (3) The microhook, that fixed the hyphal apical part.

Table 1

Electrical characteristics of the *N. crassa* apical cells

| Apical cell parameters in intact hypha | | Length of electrically isolated apical cell ($\mu$m) | Parameters of apical cell after electrical isolation | |
|---|---|---|---|---|
| $E_m$ (mV) | $R_{input}$ (M$\Omega$) | | $E_m$ (mV) | $R_{input}$ (M$\Omega$) |
| 145 | 25 | 250 | 45 | 50 |
| 140 | 15 | 150 | 110 | 80 |
| 120 | 20 | 300 | 55 | 40 |
| 135 | –[a] | 300 | 40 | – |
| 120 | – | 150 | 40 | – |
| 110 | 20 | 200 | 50 | 50 |
| 130 | 15 | 200 | 100 | 40 |

[a] The parameter was not registered

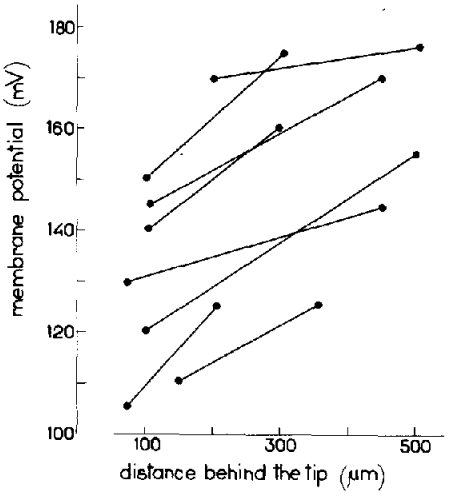


Fig.2. Membrane potential gradients of the apical zone in the intact *N. crassa* hyphae. The abscissa, the distance between the hyphal apex and the microelectrode (in $\mu$m). Parameters, recorded simultaneously with the aid of two microelectrodes in two cells of the hypha are combined by a line. The ordinate, the membrane potential value (mV).

ing 8–10 cells (800–1000 $\mu$m) did not lead to depolarization of the apical cells.

Intercellular contact resistance was calculated to be 4–6 M$\Omega$ by the method of Sokolar [13] using the $R_{input}$ mean value of an isolated apical cell (50 M$\Omega$) and the registered values of $K_c$ between the apical cells (0.65–0.85) (see [2]). Calculated values of the electrical current passing through the septal pore varied from 0.5 to 5 nA. The experiments revealed that it was not possible to obtain an electrically isolated apical cell shorter than 150 $\mu$m, i.e. the septal pore of the apical cell itself cannot be plugged. A similar phenomenon was previously observed by Trinci and Collinge [14].

## 4. DISCUSSION

Our observations revealed a membrane potential gradient in the apical zone of the fungal hyphae. Similar polarization was previously demonstrated with the aid of intracellular microelectrodes in *Achlya* hyphae [15], and by using a vibrating probe one can observe the existence of extracellular currents [5–7]. It should be stressed that in *N. crassa* these currents were estimated as proton currents [6,16].

The value of a proton current necessary for the normal function of a single cell (100 $\mu$m long and 20 $\mu$m in diameter) can be calculated as 3–10 nA taking into account that the plasma membrane $H^+$-ATPase of *N. crassa* generates an electric current of 5–20 $\mu A/cm^2$ [17].

The fact that experimentally obtained values of the intercellular currents were in agreement with the value of the proton current supporting ionic homeostasis of a single cell indicates the absence or inactivity of the $H^+$-ATPase in the apical cell. This conclusion is supported by experiments on electrical isolation of the growing apex. Depolarization up to 40–60 mV, coupled with the increase of the $R_{input}$ value of the apical cell is certainly connected with the blockade of the current, passing through the septal pores and with the disappearance of the 'metabolic component' of the membrane potential. Similar depolarization can be observed under a cyanide blockade and the exhaustion of the intracellular ATP pool (the substrate of $H^+$-ATPase) in the *N. crassa* hyphae [17,18].

Thus, the apical cells of the hyphae electrically coupled with other hyphal compartments maintain high $E_m$ levels due to the intercellular currents which serve as an energy transferring mechanism to the apical cell from the trunk. It was reported previously that secondary transport systems in *N. crassa* employ the energy of electrochemical proton gradients to drive glucose/proton symport as well as the transport of phosphate and ammonium ions [20]. The energy demands of the secondary transporters of the hyphal apical cells are probably met up by endogenous ATP in the proximal cells.

Plasma membrane ATPase of fungal cells that consumes 25–50% of cell ATP [17], competes with the mechanisms of biosynthesis and intracellular movements to obtain the ATP molecules. The release of the latter systems in the actively growing hyphal apex from the competition to obtain the ATP molecules may promote the intensity of hyphal growth. Such cooperation can exist only if an effective electric coupling with the trunk is established and the intercellular currents passing through the septal pore are comparable with the ion fluxes generated by the primary ionic pump. A study on the significance of the transmission of energy in the intercommunicating tissue systems is an interesting field for future investigation [21].

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