Ionic Currents Generated by Tip Growing Cells

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A new mechanism for the generation of ionic currents around tip growing cells is presented. Because of the absorption of a nutrient by the cell tip, its concentration decreases along the cell sides. As a nutrient molecule enters the cell with a well defined number of protons via a channel called a symport, the influx of protons decreases when one moves away from the tip. Then the balance between influx and efflux of protons is broken and a proton current loop is generated, entering the cell tip and leaving farther back. Using typical values for symport membrane current, *p*H, and nutrient concentration, we obtain an order of magnitude of the current intensity in agreement with the vibrating electrode measurements. [S0031-9007(97)03421-2]

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As many physical shapes growing with fingers or dendrites [1–3], tip growing cells such as fungal cells (hyphae) or pollen tubes, present common properties of self-organization [4]. One of these is a ionic current flowing across the cell, looping back through the external medium [5,6]. The resulting electric field can be involved in morphogenetic events [7], making a possible feedback between shape and field as many pattern-forming systems.

Extracellular currents have been measured precisely in many systems, using the vibrating electrode, especially in *fungi*. Hyphae generally drive a current of around $1\ \mu\mathrm{A/cm}^2$ of positive charges, entering the growing tip and leaving farther back. In *Achlya bisexualis*, the electric current has been ascribed to a flux of protons largely influenced by methionine concentration and *p*H [8]. In *Neurospora crassa*, protons still play a major role with several cotransported substances [9]. These phenomena present sufficiently common characteristics to validate a quantitative analysis.

Previous theories on ionic currents focus on simple cell shapes (spherical zygotes of brown algae, cylindrical shapes of characean algae) so that the ionic current pattern breaks the initial cell symmetry via an instability [10–13]. However, the conditions required for these instabilities cannot be satisfied for the tip growing cells considered here: Electrophoresis is too slow compared with the hyphal growth rate to sustain an instability [11,14], and anomalous dependence of ion fluxes with concentrations [12] is not observed for the proton-methionine system.

We propose here a mechanism which simply reflects the tip geometry of the cell. These cells maintain their large membrane potential with the proton pump H^+-ATPase which expels protons outside the cell. According to the chemiosmotic theory of Mitchell [15], the proton gradient so generated is used by the cell to absorb several substances like aminoacids and sugars via symports with protons [16]. In nonequilibrium conditions, the balance between the different proton channels does not hold, and a transmembrane proton flux J_{H^+} results. Pumps and symports are incorporated in the plasma membrane probably mainly at the tip where the growth processes are more effective. Then, if electrophoretic effects are neglected [14], they remain at rest in the frame of the membrane, and simply drift away from the tip, keeping a constant ratio between their concentrations. The diffusible morphogen or nutrient, cotransported with protons in the cell, is continuously absorbed along the hyphal trunk, resulting in a concentration gradient from tip to base [17]. Because of this external gradient, and although the concentration of channels is supposed uniform, the symport activity increases at the apex, leading to a net influx of protons at the tip and an efflux at the base and thus a proton current loop.

Let us describe this model in more detail. For simplicity we limit the dynamics to stationary currents in the extracellular medium, so that the inner concentrations will be assumed uniform. The main following species are considered: the proton H^+ and the morphogen X assumed neutral. Thanks to rapid acido-basic buffering reactions which occur near the membrane, via a buffer AH/A^- of acidity constant K_a, the *p*H is almost uniform in the bulk medium (see, for instance, experiments on *Achlya bisexualis* [8]). Similarly, because the Debye length is very small compared to the dimensions of the cell, space charges are localized close to the membrane and electroneutrality is satisfied in the bulk through a counterion Y (charge z_Y). The characteristic time scale of growth being much larger than the diffusive time scale, we neglect advective effects compared to diffusion at the cell scale. It is well known that an electric field tangential to a charged surface induces, by the intermediate of the space charged layer, an electro-osmotic flow in the bulk [18]. In the present case, the osmotic flow generated by the tangential component of the electric field around the tip growing cell is $v_e \approx 1\ \mu\mathrm{m/min}$ [19], smaller than the hyphal growth rate and is therefore neglected. Finally, if the gradients of concentration of the charged species and potential are small, the dynamical equations for the currents in the bulk

can be simplified to

$$\Delta c_X = 0, \qquad \Delta \Psi = 0\,, \tag{1}$$

where $\psi = (e/kT)\Phi$ is the dimensionless electric potential, and c_X the morphogen concentration.

At the scale of the dimensions of the cell, boundary conditions must be applied on the external cell surface, the thicknesses of the Debye and acido-basic layers being negligible. Across these layers, the total normal flux $j_{H^+} - j_{OH^-} - j_{A^-}$ is conserved. By using acido-basic constants, it can be written in the bulk only as a function of the normal gradient of Ψ [20]. At the membrane, this flux is simply J_{H^+} given by channels activity [see below, Eq. (4)], if we assume that j_{OH^-} and j_{A^-} are negligible. With the conservation of the flux of counterion and morphogen across the Debye and acido-basic layers, and the electroneutrality in the bulk, one deduces

$$C_0 \frac{\partial \Psi}{\partial n} = -\frac{J_{H^+}}{D_{\text{eff}}}\,, \tag{2}$$

$$\frac{\partial c_X}{\partial n} = -\frac{J_X}{D_X}\,. \tag{3}$$

Here $C_0 \approx b_0/2 + z_Y^2 c_{Y0}$ represents in fact the total ionic concentration [21] where b_0 and c_{Y0} are, respectively, the total buffer concentration and the counterion concentration, and D_{eff} is the diffusion coefficient of the charged species involved in acido-basic reaction, i.e., essentially A^-. For usual permeabilities and ionic concentrations, the variations of the counterion current along the hypha due to the observed potential variations appears negligible compared to the variations of the H^+ current. Consequently, J_Y has been neglected in Eq. (2). It follows that the normal component of the electric field is proportional to the local H^+ membrane current, consistent with the ion substitution experiments which conclude that the electric current is essentially carried by H^+. Contrary to a perfectly conducting body for which the normal component of the electric field at the surface is given by the local surfacic density of charges, it is given here by the local proton transmembrane flux. Thus, the electric field around a growing apex reflects almost directly the proton channels distribution and efficiency.

Then it remains to define the constitutive relations giving the fluxes of X and H^+ crossing the cell membrane. Neglecting the dependence on membrane potential, which weakly changes along the cell, we have the local fluxes

$$\begin{aligned} J_{H^+} &= J_{\text{pump}} + nJ_{\text{symp}}(c_X) + J_{\text{pass}}\,, \\ J_X &= J_{\text{symp}}(c_X)\,, \end{aligned} \tag{4}$$

where J_{pump} is the proton pump efflux (depending on pH), J_{symp} the symport influx, with a stoichiometry nH^+/X, and J_{pass} a passive current for H^+. The symport current is taken in a first approximation as $J_{\text{symp}} = -kc_X$, where k is a positive constant depending on pH, and c_X can thus be determined independently. This symport plays the major role in the mechanism: It couples the morphogen field with the potential through proton influx. Additionally, it is necessary to consider the passive flux J_{pass} since, for instance, for Achlya [8], the removal of the substance X from the culture medium inhibits the currents. Thus, in the absence of symport influx, the pump equilibrates the passive current, and an equilibrium membrane potential will result. On the contrary, in our nonequilibrium configuration, the local charge current does not vanish. Of course, the global charge conservation holds:

$$\int_\Gamma J_{H^+} dl = 0\,, \tag{5}$$

where the integral is calculated along the hyphal contour Γ at this scale. For simplicity of the analysis, the passive flux is approximated by a constant, adjusted to satisfy Eq. (5).

Taking experimental values for biological parameters, the problem is to determine whether the gradient of morphogen X along the hypha is sufficient to induce the modulation of proton influx leading to currents of $1\ \mu\text{A/cm}^2$. Because of the two different length scales of the tip shape (diameter and length), the analytical approach is delicate. Although hyphal growth is axisymmetric, we compute numerically the fields c_X and Ψ, for simplicity in two dimensions by using a boundary element method based on complex function theory. The solution is first calculated on the boundaries of the domain and then reconstructed in the bulk using the residue formula. We expect that the 2D geometry does not alter the order of magnitude of the different quantities.

The symport current has been studied in *Neurospora crassa* and presents a stoichiometry $n \approx 2$ [22], and a constant $k \approx 10^{-6}$ m/s for standard pH [23]. We use a typical value for the proton pump $J_{\text{pump}} = 10^{-6}$ mole/m^2 s [22].

The cell geometry is well defined by an hyphoid curve [24] $y = x\cot(x/d)$, where $d = 5\ \mu$m (hyphal diameter $2\pi d$), of length $L = 1$ mm, on which boundary conditions (2) and (3) are applied. On the curved part of the external contour (Fig. 1), $\Psi = 0$ and $c_X = c_{X0}$ and on the straight parts; in the rear of the cell the normal gradients of both quantities are taken equal to 0.

The numerical values defining the external medium are taken from experiments [8], where a hyphal tip of *Achlya bisexualis* grows at an almost constant elongation rate in a mixture of aminoacids, in particular, methionine of concentration $c_{X0} = 0.2$ mM, at external pH adjusted to $p\text{H} = 6.5$, with a buffer of $p\text{K}_a = 6.8$. We take $b_0 = 2$ mM and $c_{Y0} = 10$ mM [21]. Diffusion coefficients are taken as $D_{A^-} = D_X = 10^{-5}$ cm^2/s [25].

As shown in Fig. 1, the usual ionic currents pattern appears in the medium, with current lines leaving trunk and entering the growing apex, as observed in experiments [8,26]. Consequently, tip and tail potentials become, respectively, slightly negative and positive, as illustrated

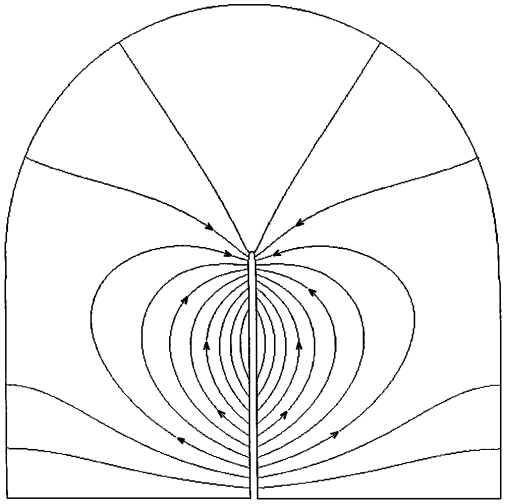

FIG. 1. Current pattern around the hyphal contour of diameter 30 μm and length 1 mm.

by the current lines which flow from the external contour to the tip or flow from the tail to the external contour of zero potential.

In Fig. 2 is shown the variation of the component of the current density transverse to the hyphal axis as a function of the curvilinear coordinate along the hypha (quantity in general reported in experimental papers), for a resistivity of the extracellular medium $\rho \approx 1000\ \Omega$ cm [8,26]. The current enters the cell at a distance of 400 μm, with a sharp variation close to the tip owing to the cell geometry, and leaves from more distal regions with correct intensity [8,26]. The general aspect of the curve is, for instance, very similar to Fig. 2A of Ref. [26].

Magnitude of tip depolarization is evaluated as $\Delta\Psi = 10^{-3}$, or $\Delta V = 0.03$ mV along the shape, for a cell of 1 mm long. As already noticed [7], this depolarization is much smaller than the one measured with intracellular electrodes, but an essential source of the cytoplasmic field [27].

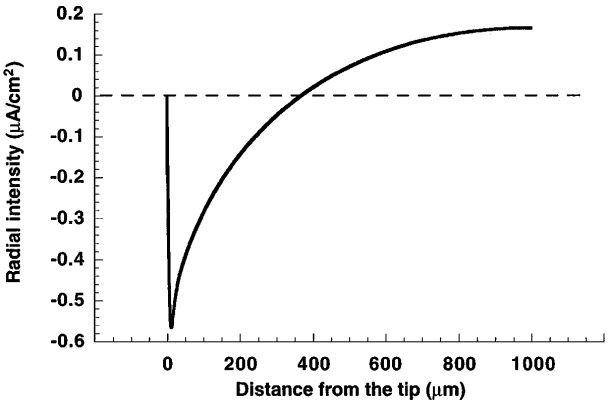

FIG. 2. Radial current intensity as a function of the distance from the tip, along the shape.

As we derived Eqs. (2) and (3), we evaluate the normal component of the proton gradient at the membrane as

$$\frac{\partial c_{\mathrm{H}^+}}{\partial n} = -\frac{z_Y^2 c_{Y0}}{C_0}\frac{J_{\mathrm{H}^+}}{D_{\mathrm{eff}}}. \tag{6}$$

By comparison with Eq. (2), we deduce $\Delta p\mathrm{H} \approx 4(c_{Y0}/b_0)\Delta\Psi \approx 0.02$, tip more alkaline, consistent with experimental observations [26] and validating our assumption of almost uniform *p*H.

When the nutrient concentration c_{X0} is reduced in our model, the currents decrease linearly with c_{X0}, simply because the symport flux is assumed to vary linearly with concentration. However, this decrease is smaller in experiments [26], probably because the cotransport kinetics is of the Michaelis-Menten type [28]. When the *p*H is increased, our model predicts that the currents almost vanish (as is observed in *Achlya bisexualis* [8]), because the prefactor $k(c_{\mathrm{H}^+})$ of the symport current decreases with lower H^+.

This model uses some aspects of Mitchell's chemiosmotic theory to tip shaped cells in nonequilibrium conditions. In order to absorb nutrients and morphogens, the cell expels protons by pumps which reenter the cell via symports. When such a mechanism occurs on a tip shaped cell, a nutrient concentration gradient is produced and a proton loop generated. Thus the electric current pattern usually observed around growing tips reflects the gradients of different substances. As shown, for instance, in the chemotropism experiment with methionine [29], the cell growth is oriented by the gradient.

The structure of diffusing fields around tip growing cells described here appears to be in close analogy with the structures of solute field around growing crystalline dendrites or metallic aggregates grown by electrodeposition. The main difference is that, in physical cases, field is almost uniform on the shape, the growth velocity being essentially sensible to the normal gradient. Here a tangential gradient is generated along the membrane which can be used by the cell to elongate differently at the tip and at the base, sustaining the tubular shape.

The mechanism proposed here can be tested experimentally by modification of the cell environment able to affect the diffusive layers, namely, imposed electric fields, concentration gradients, and external flows. The model could also be extended to other tip growing organisms, like pollen tube, with K^+ or Ca^{2+} playing the role of the nutrient, or neuronal growth cone. In this last case, the morphogens could be growth factors or neurotransmitters and the mechanism studied here, as one already proposed [30], could take part in the depolarization of neurite tips [31].

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