J. Membrane Biol. 101, 33–41 (1988)

The Journal of
Membrane Biology

Transcellular Ion Currents and Extension of *Neurospora crassa* Hyphae

Yuko Takeuchi,* Jan Schmid, John H. Caldwell,† and Franklin M. Harold‡

Department of Molecular and Cellular Biology, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206, and †Departments of Physiology and ‡Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver, Colorado 80262

Summary. Hyphae of *Neurospora crassa*, like many other tip-growing organisms, drive endogenous electric currents through themselves such that positive charges flow into the apical region and exit from the trunk. In order to identify the ions that carry the current, the complete growth medium was replaced by media lacking various constituents. Omission of K^+ or of phosphate diminished the zone of inward current, effectively shifting the current pattern towards the apex. Omission of glucose markedly reduced both inward and outward currents; addition of sodium azide virtually abolished the flow of electric current. Growing hyphae also generate a longitudinal pH gradient: the medium surrounding the apex is slightly more alkaline than the bulk phase, while medium adjacent to the trunk turns acid. The results suggest that *Neurospora* hyphae generate a proton current; protons are expelled distally by the H^+-ATPase and return into the apical region by a number of pathways, including the symport of protons with phosphate and potassium ions. Calcium influx may also contribute to the electric current that enters the apical region. There seems to be no simple obligatory linkage between the intensity of the transcellular electric current and the rate of hyphal extension. Calcium ions, however, are required in micromolar concentrations for extensions and morphogenesis of hyphal tips.

Key Words electric current · protons · *Neurospora crassa* · pH gradient · H^+-ATPase · calcium

Introduction

Fungal hyphae are highly polarized organisms that extend by incorporating new cellular material at the extreme tip. Precursors for the biosynthesis of new cell wall and plasma membrane are produced in the endoplasmic reticulum all along the hypha, packaged into vesicles in Golgi equivalents, transported vectorially to the apex and exocytosed there. The anatomy and physiology of hyphae reflect their polarized mode of extension: the shape of the tip, the chemical composition of the wall, the distribution of cytoplasmic organelles and of cytoskeletal elements, all vary in a regular manner with distance behind the tip (reviews: Grove, 1978; Gooday, 1983; Wessels, 1986).

Polarity also manifests itself in the electrical characteristics of fungal hyphae. Twenty-five years ago Slayman and Slayman (1962) observed that the membrane potential at the tip of *Neurospora* hyphae was less negative than that along the trunk; their data implied that a substantial electric current flows into the tip. Such transcellular electric currents became experimentally accessible with the development of the vibrating probe (Jaffe & Nuccitelli, 1974), an ultrasensitive extracellular electrode capable of registering the minute voltages generated by single cells and small organisms in the surrounding medium (reviews: Jaffe & Nuccitelli, 1977; Nuccitelli, 1982, 1986). We now know that many fungi drive electric currents through themselves, such that positive charges enter the apical region and exit distally (Gow, 1984). Thus far, however, only in the water mold *Achlya bisexualis* has the genesis of the current been examined in detail. *Achlya* hyphae drive a proton current through themselves; protons are expelled distally by a proton-translocating ATPase and enter the apical region by symport with amino acids (Kropf et al., 1984; Gow, Kropf & Harold, 1984; Kropf, 1986; W.J.A. Schreurs & F.M. Harold, *in preparation*).

A major impetus for research in this field comes from the widespread belief that currents are not only manifestations of cellular polarity, but may be causally involved in generating and maintaining this polarity (Jaffe & Nuccitelli, 1977; Nuccitelli, 1982, 1986). The ascomycete *Neurospora crassa* should be a suitable subject for inquiry into this relationship, thanks to the wealth of background informa-

* *Present address:* Department of Biology, College of General Education, Osaka University, Osaka 560, Japan.

tion available concerning the physiology, biochemistry and genetics of this common fungus. We report here that *Neurospora* hyphae generate an edogenous transcellular proton current. As in *Achlya*, the driving force is probably proton extrusion by the H^+-ATPase of the plasma membrane. Protons flow into the apical region, possibly by symport with various ions including phosphate and potassium. Calcium ions may carry part of the current into the hyphal apex and appear to be specifically involved in its extension. By contrast, there is no clear correlation between the rate of extension and the flow of electric charge through the hypha.

Materials and Methods

Organisms and Growth Media

Neurospora crassa wild-type strain RL21a was obtained from Dr. C.L. Slayman. Cultures were maintained on Vogel's medium; conidia, grown on slants of Vogel's medium containing 2% agar, were transferred monthly (Davis & de Serres, 1970; Slayman & Slayman, 1979).

Mycelium was grown on two media. PYG medium is a rich, complex medium containing (per liter) 1.25 g bacto peptone, 1.25 g yeast extract and 3 g glucose; acidity was adjusted to pH 6.0 with HCl. Hyphae attained a diameter of 8.1 ± 1.9 μm and extended at a rate of 10.9 ± 2.0 μm/min (n = 34, SD). The resistivity of PYG medium is 2500 ± 200 Ωcm. T medium is a modification of Vogel's medium, designed to simplify the ionic composition and to increase the resistivity; it is not an optimal growth medium. T medium contains 1 mM KH_2PO_4, 1 mM NH_4NO_3, 0.5 mM $MgSO_4$, 0.5 mM $CaCl_2$, trace metals as in Vogel's medium, 5 μg/ml biotin and 1 mM MES buffer (2[N-morpholino] ethanesulfonic acid); the pH was adjusted to 6 by addition of Tris. Glucose (17 mM) served as the source of carbon and energy; the osmolarity was adjusted to 50 mOsm by addition of sorbitol (25 mM in the case of the standard medium). Sorbitol was chosen because, unlike mannitol, it did not support growth. The average diameter of hyphae on T medium was 7.2 ± 0.9 μm, and the extension rate at pH 6 was 8.6 ± 3.2 μm/min (n = 13, SD). The resistivity of T medium is about 2000 Ωcm.

Whenever one medium was exchanged for another, the osmolarity was kept constant by the addition of sorbitol. In a few experiments T medium was supplemented with a mixture of amino acids (Kropf et al., 1984).

Preparation of Hyphae

Conidia were plated on PYG or T medium solidified with 1% agarose (BioRad Standard—low M_r), and incubated overnight at 24°C. Ordinary agar was avoided because it contains substances that stimulate growth. Next morning a rectangular block was cut from the edge of the growing colony, 3 × 5 mm and 2 mm thick. The block was attached to the center of a coverslip (35 × 50 mm) with Vaseline (Vaseline was judged superior to Silastic Elastomer used for earlier work because it does not release acid into solution). The upper surface of the agar block was covered with a fragment of coverslip (5 × 6 mm), again attached with Vaseline. The coverslip bearing the agar block was then placed in the bottom of a Lucite measuring chamber (diameter 2 cm) and covered with growth medium. Hyphae growing off the edge of the agar block were mapped as described below. When desired, media were exchanged by continuous flow at about 5 ml/min.

Vibrating Probe

The electric currents generated in the surrounding medium were mapped with a vibrating probe, an instrument capable of detecting a potential difference of 10 nV between two points 30 μm apart. Given the resistivity of the medium, the voltage reading can be converted to the current density by the application of Ohm's Law.

The instrument was constructed to the original design (Jaffe & Nuccitelli, 1974), modified according to Jaffe & Walsby (1985). The microelectrode was fabricated from stainless steel insulated with Parylene-C (SS300305A, Micro Probe, Clarksburg, MD). The tip, a 5 to 10 μm length of exposed metal, was plated successively with gold [0.2% $AuK(CN)_2 \cdot 2H_2O$] and with platinum black (1% $PtCl_2$ in 0.01% lead acetate), producing a ball of platinum black 30 μm in diameter. The reference electrode, a glass-insulated silver/silver chloride wire (In Vivo Metric, Healdsburg, CA), was positioned 3 mm from the measuring electrode. The probe was vibrated perpendicular to the hyphal axis at a frequency of 200 to 300 Hz. The voltage signal was amplified 50× before passing into the lock-in amplifier.

When the probe was situated just adjacent to the hyphal tip, the vibration caused the tip to bend towards the probe and also to branch; no such difficulties were encountered 30 μm or more behind the tip. Current densities at the tip itself were somewhat lower than those at 30 μm, but could not be measured reliably.

Mapping Extracellular pH

The pH along the hyphal surface was measured with commercial pH microelectrodes (MI-102, Microelectrodes, Londonderry, NH), essentially as described by Gow et al. (1984). The pH-sensitive tip was 50 μm in length, and the response-time about 5 sec. The buffering capacity of the medium was reduced by using 0.5 mM MES in these experiments.

Other Methods

Ammonia production or consumption was monitored in batch cultures by use of a selective electrode (MI-740, Microelectrodes). Radial colonies were grown on PYG agar plates. A small block (5 × 5 × 2 mm) was cut from the edge of a colony and incubated in PYG medium overnight, to let the hyphae proliferate. The block was then rinsed and placed in a small volume of test solution to assay NH_3 production. Media were prepared with water that had been passed over a cation exchange column (Dowex-50, protonated form) to reduce the NH_3 level below 10 μM. Calcium concentrations in the standard growth media were determined with a calcium electrode and an Orion Ionalyzer. Concentrations below 1 μM were set by addition of EGTA [ethyleneglycol-bis-(β-amino ethyl ether)-N,N'-tetraacetic acid] and estimated by use of a computer program based on Caldwell's (1970) dissociation constants.

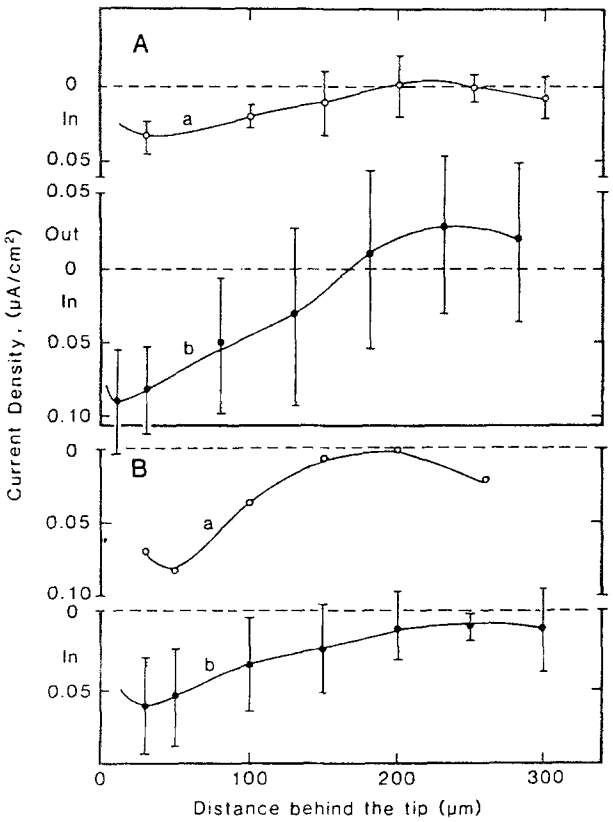

Fig. 1. Spatial patterns of transcellular electric currents generated by *Neurospora* hyphae. (*A*) In PYG medium, pH 6.0. (*a*) An individual hypha was mapped 5 times over the span of an hour. (*b*) Average map from many hyphae, 20–40 individuals for each point. (*B*) In T medium, pH 6.0. (*a*) Map of an individual hypha. (*b*) Average of multiple hyphae, 7–23 depending on position. Bars represent SD. Currents at the tip itself were smaller than just behind the tip, but could not be measured accurately

Results

The Transcellular Electric Current

Patterns of electric current generated by growing hyphae were mapped in both the rich, complex PYG medium and in the lean, defined T medium. Figure 1*A*, trace *a*, documents the reproducibility of the current pattern generated by an individual hypha in PYG over the span of an hour. The intensity of the current varied considerably from one hypha to another, but the spatial patterns of current flow were much alike (Fig. 1*A*, trace *b*). Hyphae grown in T medium were thinner than those from PYG, extended less rapidly and generated a weaker current of the same general configuration (Fig. 1*B*). In both media current flowed into the apical region, with the peak of inward current 20 to 50 μm behind the tip (currents at the tip itself were indeterminate for technical reasons). Inward current declined as a function of distance, reaching zero at 100–200 μm behind the tip. In hyphae grown on PYG, outward current was recorded beyond 200 μm, while hyphae grown on T medium produced no outward current as far back as 300 μm. Longer hyphae undulate too much to be mapped. Our inability to map the entire length presumably accounts for the apparent excess of inward over outward current. The current patterns in both media were much the same at pH 5 and 6, but at pH 7 currents were smaller and more variable; extension was also somewhat inhibited.

Current densities at the surface were estimated by extrapolating from measurements made at increasing radial distance (three hyphae). Assuming exponential fall-off, currents at the surface 30 μm behind the tip ranged from 0.2 to 0.4 μA/cm^2; but this estimate neglects the possibility that current density in the periplasmic space may be much higher than in the bulk medium.

Which Ions Carry the Current?

Ions that contribute significantly to the current can often be recognized from the effects of ion substitution. These experiments were performed with hyphae grown on T medium, which was designed for the purpose.

Omission of $MgSO_4$, NH_4NO_3, MES, biotin and trace elements had no consistent effect on the current pattern (omission of the nitrogen source did, however, considerably reduce the rate of extension). Current and growth were also unaffected by modifications to make chloride the sole anion, or to substitute nitrate for chloride. Addition of amino acids and vitamins to T medium did not alter the current pattern (*data not shown*). The only constituents whose omission had significant effects were potassium, phosphate, calcium and glucose.

When T medium was replaced by a modified medium containing Na^+ in place of K^+, the current pattern shifted anteriorly: outward current, normally seen only 200 μm or more behind the tip, appeared at about 100 μm (Fig. 2*A*). Simply removing K^+ from T medium had a similar effect, albeit less marked. Hyphae growing in the absence of K^+ were thin, sinuous and extended at a reduced rate, in keeping with the common presumption that potassium ions help maintain turgor. The drug 3,4-diaminopyridine (100 μM), known to block potassium channels in animal cells, mimicked the effects of K^+ removal on currents and growth (*data not shown*); however, we were unable to demonstrate inhibition of net K^+ uptake by 3,4-diaminopyridine.

Substitution of a medium lacking phosphate also shifted the current pattern anteriorly (Fig. 2*B*),

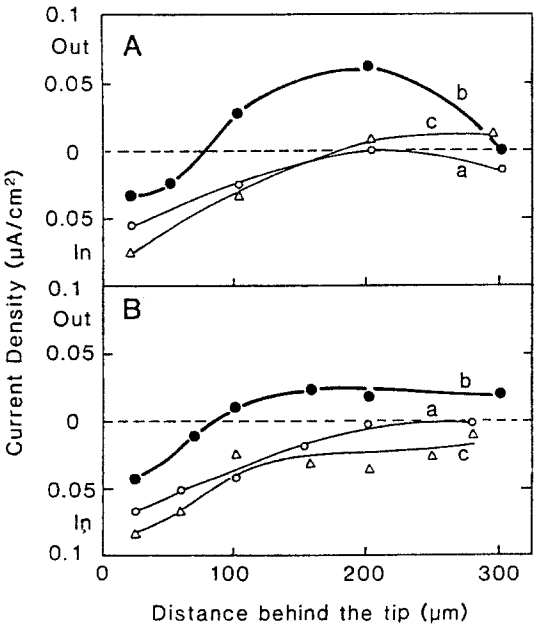

Fig. 2. Ion substitutions alter the current pattern. (*A*) Potassium. (*a*) Map of a hypha growing in T medium, rate 6 μm/min. (*b*) The medium was replaced by a modified one containing Na^+ in place of K^+. The map was measured 7 min after the beginning of exchange; extension rate, 4 μm/min. (*c*) Restored T medium, map was measured after 7 min. Extension rate, 5 μm/min. (*B*) Phosphate, procedure as in *A*. (*a*) Hypha growing in T medium, rate 6 μm/min. (*b*) After replacement of medium by one lacking phosphate; extension rate, 4 μm/min. (*c*) After restoration of T medium; extension rate 3 μm/min

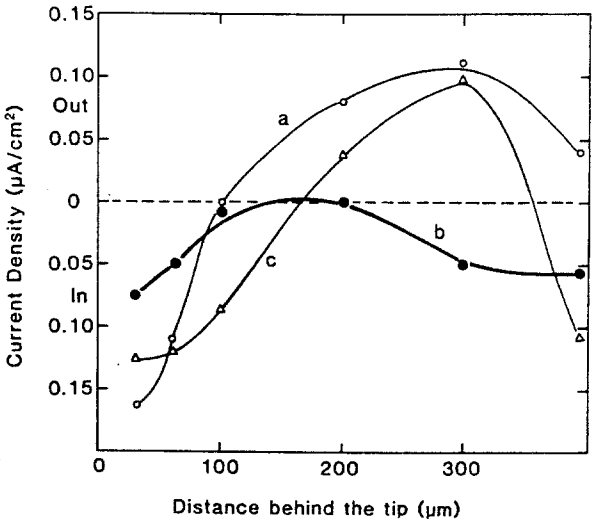

Fig. 3. The current pattern depends on glucose. (*a*) Map of a hypha growing in PYG medium, rate 8 μm/min. (*b*) Eleven min after replacement of glucose by sorbitol; extension rate, 6.5 μm/min. (*c*) Eight min after restoration of glucose; extension rate, 8 μm/min

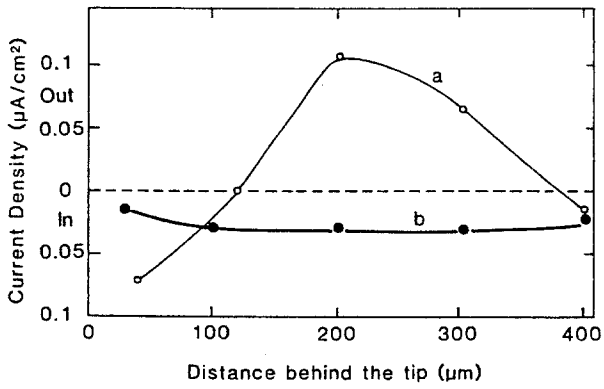

Fig. 4. Azide abolishes the current. (*a*) Map of a hypha growing in PYG medium at 8 μm/min. (*b*) Ten min after replacement of medium by PYG plus 1 mM NaN_3; extension had ceased

and diminished both the width of the hyphae and their rate of extension. We interpret the findings to suggest that the uptake of both K^+ and phosphate contributes to the flow of positive charges into the apical region.

The most dramatic effects were seen upon omission of glucose, the sole source of carbon and energy in T medium and their chief source even in PYG. As shown in Fig. 3, both the inward and outward current limbs were greatly diminished but a small inward current persisted at the apex. Hyphal diameter decreased by half but the hyphae continued to extend, sometimes at the normal rate (10 μm/min). Both growth and current recovered within a few minutes after the restoration of glucose. Results in PYG and T media were essentially the same. (Unexpectedly, when glucose was replaced by the nonmetabolizable monosaccharide 3-O-methylglucose, hyphae grew thin as in the absence of glucose, but the flow of current was unaffected or even stimulated. We have no convincing explanation for this finding).

The dependence of the current on metabolism was confirmed by the inhibitory effects of 1 mM NaN_3 (Fig. 4). Growth stopped completely and the current was abolished, except for a tiny residual influx of charge into the apical region. When NaN_3 was washed out, currents and growth recovered partially. Taken all together, the results suggest that the transcellular electric current represents chiefly a flow of protons. These are expelled distally by the H^+-ATPase and return into the hyphal anterior by several pathways, including symport of protons with phosphate and K^+ ions.

A Longitudinal Gradient of Extracellular pH

If hyphae drive a current of protons through themselves, one may expect to find the medium sur-

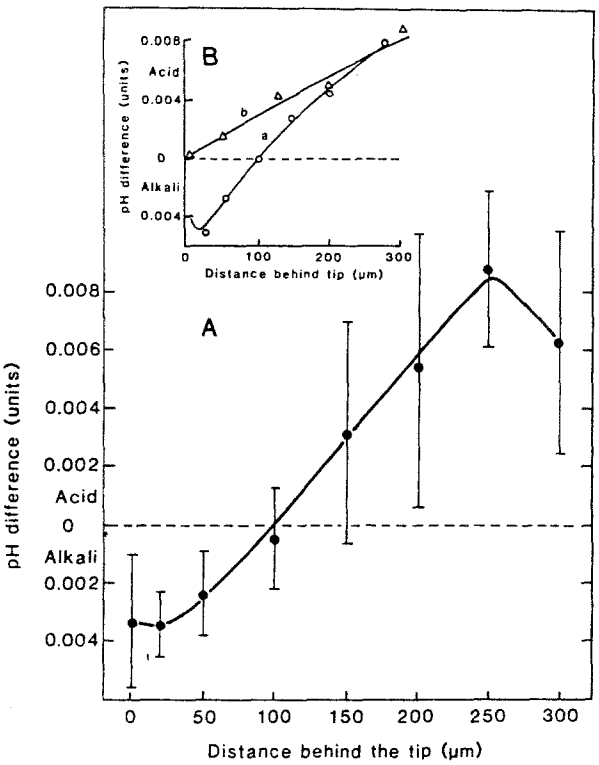

Fig. 5. Growing hyphae generate a longitudinal pH gradient in the surrounding medium. (*A*) Average pH map from 20 individual hyphae growing in PYG medium, pH 6.0; bars represent SD. (*B*) Inset: pH maps of individual hyphae in PYG (*a*) or T medium (*b*) at pH 6.0

rounding the apical region to be relatively depleted of protons, and therefore slightly alkaline; by the same token, the distal region of net proton extrusion should be relatively acidic. This expectation was realized in hyphae grown on PYG medium: mapping with extracellular pH microelectrodes revealed an alkaline zone around the tip and back as far as 200–300 μM, while more distal portions of the hypha generated acid (Fig. 5*A*). Hyphae grown on T medium had no frankly alkaline zone around the tip, but acid production was much less pronounced at the apex than along the trunk (Fig. 5*B*). Addition of amino acids and vitamins did not alter the pH profile.

The acidic zone along the trunk must primarily represent the efflux of acidic metabolites; part of the acid arises from glucose metabolism, since removal of the sugar diminished acid production and lengthened the alkaline zone (*data not shown*). The alkaline zone may represent proton influx, but we cannot exclude the possibility that it is due to ammonia production. Experiments on batch cultures showed that hyphae take up NH_3 from both T and PYG medium. However, in the absence of microelectrodes capable of monitoring NH_3 levels along the hyphal surface, we must leave open the possibility that hyphae in PYG medium generate ammonia, which is subsequently consumed. In sum, the extracellular pH profile reflects the dynamic interplay of several localized processes including fluxes and metabolic reactions.

Do Calcium Ions Contribute to the Current?

Neurospora hyphae grow well on PYG medium which contains only 8 μM free calcium ions, and drive a well-defined transcellular current (Fig. 1). However, further reduction of the calcium concentration induced changes in the pattern of extension as well as electric currents. For example, when T medium (500 μM Ca^{2+}) was replaced by medium lacking any added calcium (concentration of free Ca^{2+} about 2 μM), hyphae continued to extend and appeared morphologically normal but the flow of transcellular electric current was consistently reduced. When sufficient EGTA was added to reduce the concentration of free calcium ions below 1 μM, extension slowed to a half or a third of the original rate, and many hyphae put forth apical branches or bulbous swellings. The current patterns fluctuated wildly; on some occasions we saw large inward currents that probably report injury to the tip (*data not shown*).

There is reason to believe that calcium ions are involved specifically in apical extension. When mass cultures of *Neurospora* hyphae were grown overnight in calcium-deficient medium (<1 μM free Ca^{2+}) the yield of mycelial mass was only slightly reduced and its content of protein and RNA was normal. However, the hyphae were unusually wide, misshapen and less than half the length of controls (*data not shown*).

Must calcium ions pass through specific channels? Lanthanum and gadolinium ions, which effectively block calcium channels in *Blastocladiella* (Caldwell, van Brunt & Harold, 1986), had no obvious effect on hyphae extension or on the pattern of branching. Nifedipine, known to block calcium channels in animal cells, partially inhibited extension of *Neurospora* hyphae at 100 μM and distorted the pattern of transcellular electric current, but did not elicit branching or other abnormalities. The effects of nifedipine were not reversed by raising the extracellular calcium level, and we suspect that they have nothing to do with ion channels. There is thus no evidence that calcium ions pass across the plasma membrane by calcium channels of the usual sort.

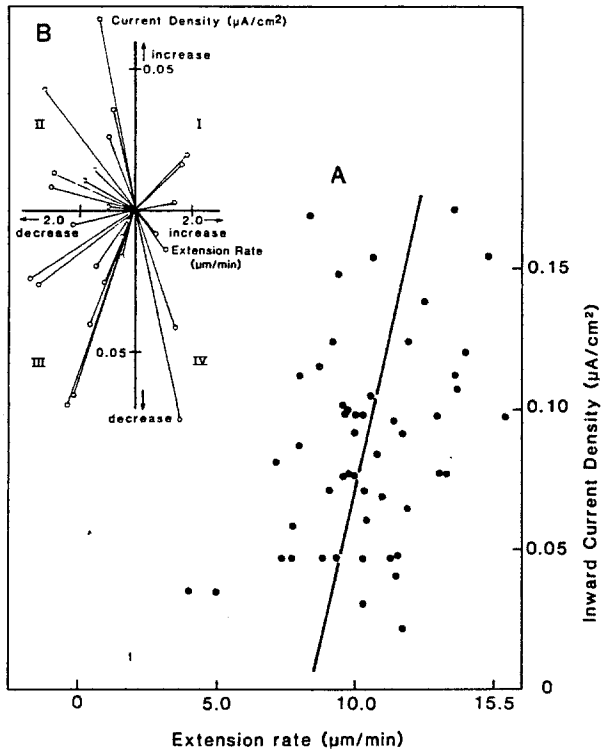

Fig. 6. Relationship of current intensity to growth rate. (*A*) Data from numerous hyphae growing in both PYG and T media were pooled. For each hypha, the peak inward current density is plotted against extension rate. The line represents the best fit from a regression analysis, correlation coefficient 0.36. (*B*) Changes in the rate of extension are not necessarily correlated with changes in current density. In any given hypha, both the rate of extension and the current density fluctuate spontaneously. These data were collected from 25 hyphae growing in PYG medium, pH 6.0. Distance along the abscissa represents the change in the rate of extension of a particular hypha, while the distance along the ordinate displays the simultaneous change in current density

Are Electric Currents Causally Related to Growth?

In *Neurospora*, as in other organisms, currents and growth tend to be correlated. As a rule, the greater the current the faster does that hypha extend, but the correlation coefficient is only 0.36 (Fig. 6*A*). Wide hyphae also generate a more intense and more stable current than do thin ones (correl. coeff. 0.49; *data not shown*). But the linkage between extension and the total ionic current is a loose one. Under our conditions, hyphae often extend in an irregular manner, by fits and starts. Figure 6*B* shows that, when the growth of a hypha slowed spontaneously, the current was as likely to intensify (quadrant *II*) as to diminish (quadrant *III*). There were also many occasions when extension spurted while inward current declined (quadrant *IV*). Extension and current could also be uncoupled by the addition of sorbose, which inhibits synthesis of the primary cell wall and induces branching (Mishra & Tatum, 1972). Addition of 0.1 M sorbose to PYG medium reduced the growth rate from 12 to 3.5 μm/min but had no effect on the intensity or the spatial pattern of the electric current (*data not shown*).

Discussion

Our present understanding of ionic relations in *Neurospora* stems very largely from the research carried out during the past two decades by C.L. Slayman, C.W. Slayman and their associates (Slayman, 1965*a,b*, 1970, 1980; Gradmann et al., 1978; Goffeau & Slayman, 1981; Sanders, Slayman & Pall, 1983; Rodriguez-Navarro, Blatt & Slayman, 1986; Blatt & Slayman, 1987). In a nutshell, these investigators showed that energy coupling at the plasma membrane is effected in a chemiosmotic manner by a circulation of protons. Protons are expelled by a H^+-ATPase, generating a protonic potential across the plasma membrane; they return to the cytoplasm by diverse routes, including an array of symporters and antiporters. The porters are involved in maintaining the stability of cytoplasmic pH and turgor, and support the net uptake of ions and other metabolites during growth. Our results provide initial insight into the spatial organization of this network of fluxes. It appears that ion pumps and porters are differentially placed along the hypha, such that a significant fraction of the protons expelled from the hyphal trunk returns into the apical region, together with K^+, P_i and possibly other ions.

The basic finding is that *Neurospora* hyphae drive an electric current through themselves, such that positive charge flows into the anterior and out of distal regions of the trunk (Fig. 1). Our proposal that the current represents a longitudinal circulation of protons modulated by additional ion fluxes, rests on two arguments: (i) Substitution experiments indicate that none of the common ions of the growth medium carry a major share of the current, making protons the favored candidate. Glucose metabolism is plainly implicated in the generation of the current (Figs. 3 and 4), which we attribute to the proton-translocating ATPase of the plasma membrane. (ii) The extracellular pH profile, such that the medium adjacent to the apex is slightly alkaline, supports the premise that protons diffuse into the apical zone. However, we cannot rigorously exclude the possibility that apical alkalinity reports the production of ammonia or some other alkaline metabolite. In the absence of evidence to the contrary, we shall adopt the parsimonious hypothesis that both the

transcellular electric current and the extracellular pH profile reflect the circulation of protons through the hypha.

Just how protons pass inward across the apical surface is not certain, but we would expect proton-coupled porters to play a role. The H^+/glucose symporter characterized by Slayman and Slayman (1974) should be repressed in our cells, which were grown on glucose. More plausible candidates are the transport systems for K^+ and phosphate. Omission of either ion from the growth medium augmented outward current and shifted it toward the hyphal tip (Fig. 2); the interpretation of this change in pattern will be considered shortly. Unfortunately, neither potassium nor phosphate transport is fully understood. *Neurospora* produces an inducible high-affinity system that mediates H^+/K^+ symport (Rodriguez-Navarro et al., 1986), but it is uncertain whether this will be active under our conditions. Phosphate uptake by symport with protons has been reported in yeast (Cockburn, Earnshaw & Eddy, 1975), and in duckweed (Ullrich-Eberius, Novacky & Van Bel, 1984), but it is not known whether this also occurs in *Neurospora*. The possible contribution of calcium influx to the current pattern will be considered below.

The longitudinal ion circulation must reflect non-uniform distribution of electrogenic transport systems along the hypha. In principle, *Neurospora* may exclude the H^+-ATPase from the apex while porters and ion channels are scattered at random; conversely, porters may be concentrated in the hyphal anterior while proton pumps are evenly distributed; finally, both distributions may be non-uniform. Our observations say little about the distribution of porters, but favor the proposition that the hyphal anterior is relatively depleted of proton pumps. The most visible feature of the extracellular pH gradient (Fig. 5) is not the faint apical alkalinity, but the generation of acid along the hyphal trunk which reflects the conversion of glucose into unidentified acidic metabolites. Several recent studies implicate the H^+-ATPase in the extrusion of acid from both *Neurospora* (Sanders, Hansen & Slayman, 1981; Sanders & Slayman, 1982; Blatt & Slayman, 1987) and yeast (Ulaszewski et al., 1987). Net acid production requires compensatory movement of cations or anions, and it is conceivable that either the metabolic enzymes or the compensatory ion fluxes are preferentially found behind the tip. However, the simplest hypothesis postulates that it is the H^+-ATPase whose distribution accounts for the gradient of acid production. The hypothesis that the apical region is relatively deficient in H^+-ATPase while porters are uniformly distributed is consistent with the finding that omission of K^+ or phosphate

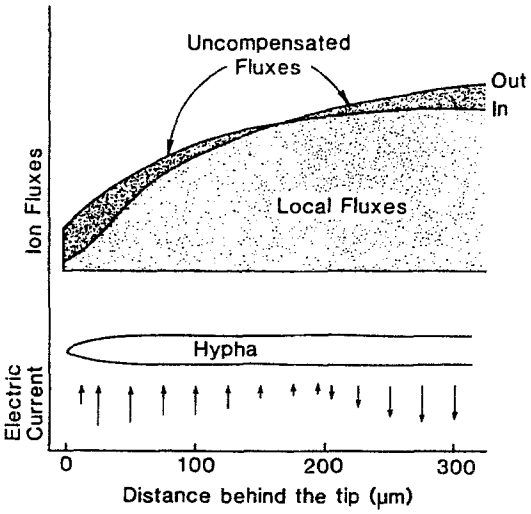

Fig. 7. Dynamic interplay of ion fluxes involved in the generation of the transcellular electric current and pH profile. The hypothesis proposes that acid secretion along the hyphal trunk reflects the distribution of H^+-ATPase. Fluxes of K^+, Na^+, phosphate and metabolic anions compensate for most, but not all, of the proton current through local circuits. The transcellular electric current and apical alkalinity reflect the nonuniform distribution of transport systems in the apical region, especially the partial exclusion of H^+-ATPase from the tip

augments outward current and shifts the current pattern anteriorly (Fig. 2), and also with the effects of glucose omission on the pH profile. Cytological studies by Jennings and his colleagues (Galpin & Jennings, 1975*a,b*) led them to infer that ATPase is excluded from the apical regions of *Dendryphiella* and *Phycomyces*; in the future it may be feasible to localize the H^+-ATPase protein directly by immunocytochemistry.

The segregation of proton pumps from metabolite porters is probably quite incomplete (Fig. 7). According to Slayman (1980) the H^+-ATPase drives a proton current of about 25 $\mu A/cm^2$ across the plasma membrane, yet less than 1 $\mu A/cm^2$ flows longitudinally through the hypha. Most of the proton circulation thus appears to be local, but the small fraction that passes longitudinally through the hypha is not necessarily negligible. Assuming a proton influx of 0.5 $\mu A/cm^2$ at the surface, a hyphal diameter of 10 μm, and a buffer capacity of 137 mM H^+/pH unit (Blatt & Slayman, 1987), one can calculate that the cytoplasmic pH in the apical region will fall by 0.1 unit every 11 min unless the protons are pumped out again elsewhere. This is a significant acid load that may have a physiological role in extension of the apex (Harold, Caldwell & Schreurs, 1987).

Of all the constituents of the transcellular cur-

rent, calcium ions may prove to be the most interesting, because of their apparent linkage to apical extension. Tip growth of diverse plant cells clearly requires Ca^{2+} ions, which may pass through special calcium channels blocked by lanthanide ions and by nifedipine (Jaffe, Weisenseel & Jaffe, 1975; Picton & Steer, 1985; Reiss & Herth, 1985; Saunders, 1986; Review: Hepler & Wayne, 1985). *Neurospora* hyphae require at least 1 μM Ca^{2+} for growth with normal morphology, 10 μM to attain the maximal extension rate. The fraction of electrical current carried by calcium ions cannot be assessed simply by chelating Ca^{2+} with EGTA, for it is well known that many electrophysiological characteristics of *Neurospora* are altered in calcium-deficient media (Slayman, 1965*a; personal communication*). Diminution of the transcellular current may thus reflect changes in the apical conductance of potassium and other ions, and does not necessarily report a massive influx of calcium ions. Attempts to detect calcium channels by the use of lanthanide ions and nifedipine were unsuccessful, leaving us in doubt whether calcium ions must cross the plasma membrane at all. Indeed, it is conceivable that the lack of external Ca^{2+}, rather than the cessation of influx, is ultimately responsible for the perturbation of apical extension. Attempts to unravel this puzzle are under way.

What, then, has the transcellular ion current to do with the polarized extension of fungal hyphae? Is the flux of ions through the apical cytoplasm, or the electric field induced by the ion current, a necessary feature of tip growth? Figure 6 reinforces the doubts expressed on previous occasions: while there is some correlation between hyphal extension and the transcellular electric current, changes in one parameter frequently do not match changes in the other. The results argue against the proposition that the electric field across the cytoplasm plays an obligatory role in hyphal extension. They would, however, be consistent with the hypothesis that the influx of protons or of calcium ions creates conditions that allow the hyphal protoplasma to extend (Harold et al., 1987).

We thank Diane Nicholl for technical assistance, Nobuo Matsusita for advice on statistics, and Dr. Alexander Scriabine of Miles Laboratories for a gift of nifedipine. Jan Schmid is a postdoctoral fellow of the Deutsche Forschungsgemeinschaft. This work was supported in part by grant GM-33354 from the National Institutes of Health.

References

Blatt, M.R., Slayman, C.L. 1987. Role of "active" potassium transport in the regulation of cytoplasmic pH by non-animal cells. *Proc. Natl. Acad. Sci. USA* **84**:2737–2741

Caldwell, P.C. 1970. Calcium chelation and buffers. *In:* Calcium and Cellular Functions. A.W. Cuthbert, editor. pp. 10–16. St. Martins, London

Caldwell, J.H., Van Brunt, J., Harold, F.M. 1986. Calcium-dependent anion channel in the water mold *Blastocladiella emersonii*. *J. Membrane Biol.* **89**:85–97

Cockburn, M., Earnshaw, P., Eddy, A.A. 1975. The stoichiometry of the absorption of protons with phosphate and L-glutamate by yeasts of the genus *Saccharomyces*. *Biochem. J.* **146**:705–712

Davis, R.H., Serres, F.J. de 1970. Genetic and microbiological research techniques for *Neurospora crassa*. *Methods Enzymol.* **17A**:79–149

Galpin, M.F.J., Jennings, D.M. 1975*a*. Histochemical study of the hyphae and the distribution of adenosine triphosphatase in *Dendryphiella salina*. *Trans. Br. Mycol. Soc.* **65**:477–483

Galpin, M.F.J., Jennings, D.M. 1975*b*. Potassium and ATPase in *Phycomyces*. *Trans. Br. Mycol. Soc.* **66**:151–153

Goffeau, A., Slayman, C.W. 1981. The proton-translocating ATPase of the fungal plasma membrane. *Biochim. Biophys. Acta* **639**:197–223

Gooday, G.W. 1983. The hyphal tip. *In:* Fungal Differentiation, A Contemporary Synthesis. J.E. Smith, editor. pp. 315–356. Marcel Dekker, New York

Gow, N.A.R. 1984. Transhyphal electrical currents in fungi. *J. Gen. Microbiol.* **130**:3313–3318

Gow, N.A.R., Kropf, D.L., Harold, F.M. 1984. Growing hyphae of *Achyla bisexualis* generate a longitudinal pH gradient in the surrounding medium. *J. Gen. Microbiol.* **130**:2967–2974

Gradmann, D., Hansen, U.P., Long, W.S., Slayman, C.L., Warncke, J. 1978. Current-voltage relationships for the plasma membrane and its principal electrogenic pump in *Neurospora crassa*. *J. Membrane Biol.* **39**:333–367

Grove, S.N. 1978. The cytology of hyphal tip growth. *In:* The Filamentous Fungi. Vol. III. J.E. Smith and D.R. Berry, editors. pp. 28–50. John Wiley, New York

Harold, F.M., Caldwell, J.H., Schreurs, W.J.A. 1987. Endogenous electric currents and polarized growth of fungal hyphae. *In:* Spatial Organization in Eukaryotic Microbes. R.K. Poole and A.P.J. Trinci, editors. Special Publications of the Society for General Microbiology (*in press*)

Hepler, P.K., Wayne, R.O. 1985. Calcium and plant development. *Annu. Rev. Plant. Physiol.* **36**:397–439

Jaffe, L.A., Weisenseel, M.H., Jaffe, L.F. 1975. Calcium accumulations within the growing tips of pollen tubes. *J. Cell Biol.* **67**:488–492

Jaffe, L.F., Nuccitelli, R. 1974. An ultrasensitive vibrating probe for measuring steady electrical currents. *J. Cell. Biol.* **63**:614–628

Jaffe, L.F., Nuccitelli, R. 1977. Electrical control of development. *Annu. Rev. Biophys. Bioeng.* **6**:445–476

Jaffe, L.F., Walsby, A.E. 1985. An investigation of extracellular electrical currents around cyanobacterial filaments. *Biol. Bull.* **168**:476–481

Kropf, D.L. 1986. Electrophysiological properties of *Achyla* hyphae: Ionic currents studied by intracellular potential recording. *J. Cell Biol.* **102**:1209–1216

Kropf, D.L., Caldwell, J.H., Gow, N.A.R., Harold, F.M. 1984. Transcellular ion currents in the water mold *Achlya*. Amino acid proton symport as a mechanism of current entry. *J. Cell Biol.* **99**:486–496

Mishra, N.C., Tatum, E.L. 1972. Effect of L-sorbose on polysaccharide synthetases of *Neurospora crassa*. *Proc. Natl. Acad. Sci. USA* **69**:313–317

Nuccitelli, R. 1982. Transcellular ion currents: Signals and effectors of cell polarity. *Modern Cell Biol.* **2**:451–481

Nuccitelli, R. (Editor) 1986. Ionic Currents in Development. A.R. Liss, New York

Picton, J.M., Steer, M.W. 1985. The effects of ruthenium red, fluorescein isothiocyanate and trifluoperazine on vesicle transport, vesicle fusion and tip extension in pollen tubes. *Planta* **163**:20–26

Reiss, H.-D., Herth, W. 1985. Nifedipine-sensitive calcium channels are involved in polar growth of lily pollen tubes. *J. Cell Sci.* **76**:247–254

Rodriguez-Navarro, A., Blatt, M.R., Slayman, C.L. 1986. A potassium-proton symport in *Neurospora crassa*. *J. Gen. Physiol.* **87**:649–674

Sanders, D.L., Hansen, U.P., Slayman, C.L. 1981. Role of the plasma membrane proton pump in pH regulation in non-animal cells. *Proc. Natl. Acad. Sci. USA* **78**:5903–5907

Sanders, D.L., Slayman, C.L. 1982. Control of intracellular pH. Predominant role of oxidative metabolism, not proton transport, in the eukaryotic microorganism *Neurospora crassa*. *J. Gen. Physiol.* **80**:377–402

Sanders, D.L., Slayman, C.L., Pall, M.L. 1983. Stoichiometry of H^+/amino acid cotransport in *Neurospora crassa* revealed by current-voltage analysis. *Biochim. Biophys. Acta* **735**:67–76

Saunders, M.J. 1986. Cytokinin activation and redistribution of plasma-membrane ion channels in *Funaria*. *Planta* **167**:402–409

Slayman, C.L. 1965*a*. Electrical properties of *Neurospora crassa*. Effects of external cations on the intracellular potential. *J. Gen. Physiol.* **49**:69–92

Slayman, C.L. 1965*b*. Electrical properties of *Neurospora crassa*. Respiration and the intracellular potential. *J. Gen. Physiol.* **49**:93–116

Slayman, C.L. 1970. Movement of ions and electrogenesis in microorganisms. *Am. Zool.* **10**:377–392

Slayman, C.L. 1980. Transport control phenomena in *Neurospora*. *In:* Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas, and J. Dainty, editors. pp. 179–180. Elsevier/North Holland, Amsterdam

Slayman, C.L., Slayman, C.W. 1962. Measurement of membrane potentials in *Neurospora*. *Science* **136**:876–877

Slayman, C.L., Slayman, C.W. 1974. Depolarization of the plasma membrane of *Neurospora crassa* during active transport of glucose. *Proc. Natl. Acad. Sci. USA* **71**:1935–1939

Slayman, C.L., Slayman, C.W. 1979. Whole cells for the study of transport linked to membrane potential: *Neurospora crassa*. *Methods Enzymol.* **55**:656–666

Ulaszewski, S., Van Herck, J.-C., Dufour, J.-P., Kulpa, J., Nieuwenhuis, B., Goffeau, A. 1987. A single mutation confers vanadate resistance to the plasma membrane H^+-ATPase from the yeast *Schizosaccharomyces pombe*. *J. Biol. Chem.* **262**:223–228

Ullrich-Eberius, C.I., Novacky, A., Van Bel, A.J.E. 1984. Phosphate uptake in *Lemma gibba:* Energetics and kinetics. *Planta* **161**:46–52

Wessels, J.G.H. 1986. Cell wall synthesis in apical hyphal growth. *Int. Rev. Cytol.* **104**:37–79

Received 4 June 1987