Electrophysiological Properties of *Achlya* Hyphae: Ionic Currents Studied by Intracellular Potential Recording

Darryl L. Kropf

Department of Molecular and Cellular Biology, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206. Dr. Kropf's present address is Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331.

Abstract. The electrical properties of the water mold *Achlya bisexualis* were investigated using intracellular microelectrodes. Hyphae growing in a defined medium maintained a membrane potential (V_m) of −150 to −170 mV, interior negative. Under the conditions used here, this potential was insensitive to changes in the inorganic ion composition of the medium. Changes in external pH did affect V_m, but only outside the physiological pH range. By contrast, the addition of respiratory inhibitors caused a rapid depolarization without affecting the conductance of the plasma membrane. Taken together these findings strongly suggest that the membrane potential is governed by an electrogenic ion pump rather than by an ionic diffusion potential.

Previous work from this laboratory showed that *Achlya* hyphae generate a transcellular proton current that enters the growing tip, flows along the hyphal length, and exits distally from the trunk. These initial experiments used an extracellular vibrating electrode, and I now report intracellular electrical recordings which support the hypothesis that protons enter the tip by symport with amino acids and are expelled distally by a proton-translocating ATPase. Most significantly, current flowing intracellularly along the hyphal length is associated with a cytoplasmic electric field of 0.2 V/cm or greater. Conditions that inhibit the current also abolish the internal field, suggesting that these two phenomena are closely linked.

Tip elongation in fungal hyphae is an excellent example of polarized growth; new plasma membrane and cell wall are deposited almost exclusively at the apex. Evidence collected over the past two decades has shown that most, if not all, tip-growing organisms generate transcellular electric currents at their apices (1, 3, 7, 20, 23, 38, 41, 42); in many cases the appearance of localized inward current precedes the emergence of a nascent tip and accurately predicts its site (20, 22, 42). Such findings suggest that electric currents play a causal role in polarized growth, but the underlying mechanism(s) is/are unclear. One possibility is that the electric field created by the current flow induces both cytoplasmic and membrane asymmetry by redistributing charged macromolecules and organelles (16). However, it has proved difficult to measure reliably the internal field owing to its small magnitude and the possibility of introducing artifacts during electrode impalement.

Previous findings from our laboratory (using an extracellular vibrating probe) showed that growing hyphae of the water mold *Achlya* generate a transcellular electric current as depicted in Fig. 1 (20). Protons carry current into the tip and we postulated that they cross the plasma membrane by symport (co-transport) with amino acids, particularly methionine (8, 19). The current entering the tip flows through the hyphal cytoplasm toward the region of outward current. We suggested that the outward current was driven by proton extrusion from an electrogenic H^+-ATPase (19). To complete the current loop, charge flows through the extracellular medium from trunk to tip. This proton circulation may be expected to make the cytoplasm at the tip acidic and electropositive with respect to the zone of outward current.

This concept of the nature and genesis of the transcellular current has now been reinforced by the use of intracellular microelectrodes. Three aspects of the model were examined in this study: (*a*) the existence of a primary ion pump in the plasma membrane, (*b*) the electrogenicity of methionine transport, and (*c*) the presence of a cytoplasmic voltage gradient near the apex. The most striking finding was the presence of an intracellular electric field of 0.2 V/cm immediately behind the hyphal tip. The intracellular electric field was abolished under conditions that block the flow of transcellular current, indicating that these two phenomena are closely linked in the physiology of this organism. Although the function of the field remains to be determined, calculations show that it is of sufficient magnitude to transport anionic cellular constituents to, and localize them at, the growing tip by self-electrophoresis.

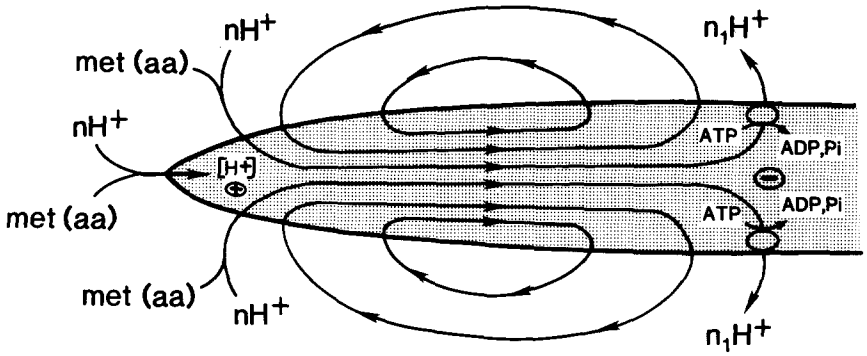

Figure 1. A model of the transcellular current generated by *Achlya* hyphae growing in DMA medium. The inward current is depicted as the symport of n protons with one amino acid molecule (*aa*), most often methionine (*met*), and the outward current is postulated to be generated by an electrogenic ATPase that expels n_1 protons per ATP hydrolyzed. The drawing is not intended to indicate localization of these transport systems; their distributions along the hyphal length is unknown. The putative gradients of pH and potential are also indicated. The [H^+] symbol indicates the relatively high proton concentration at the apex and the polarity of the cytoplasmic field is shown by + and −. See text for discussion.

Materials and Methods

Culture Conditions

The maintenance of stock cultures and preparation of zoospores of *Achlya bisexualis* T5 were described previously (19). The composition of DMA, a complete, defined medium, was as follows: K-Pipes, 1.0 mM; KH_2PO_4 and K_2HPO_4, 0.5 mM each; glucose, 10 mM. Salts: $MgCl_2$, 1.0 mM; $CaCl_2$, 0.5 mM. Trace metals: H_3BO_3, 11 μM; $MnSO_4$, 1.8 μM; $CoSo_4 \cdot 7H_2O$, 0.7 μM; $NaMoO_4 \cdot 2H_2O$ and $ZnSo_4 \cdot 7H_2O$, 0.4 μM each; $CuSO_4 \cdot 5H_2O$, 0.3 μM. Amino acids: glutamic acid, 1.36 mM; methionine, 0.22 mM; isoleucine, leucine, threonine, valine, and lysine, 0.20 mM each; glycine, arginine, phenylalanine, tyrosine, and serine, 0.10 mM each; histidine, 0.05 mM; tryptophan, 0.02 mM. The pH was adjusted to 6.5 with KOH and KCl was added so that the total K^+ added in this step was 3.0 mM. When DMA was made to lack certain nutrients, osmolarity was maintained at 28 mOsm by addition of mannitol. Whenever the pH of DMA was raised above pH 7.5, the P_i concentration was reduced from 1.0 mM to 10 μM to prevent precipitation of calcium phosphate. The resistivity of DMA was 1,000 Ω·cm.

To prepare hyphae for intracellular recording, a few hundred spores in a calcium chloride solution were pipetted aseptically onto a polycarbonate membrane filter (3-μm pores, 25-mm diameter) that had been placed on the surface of a DMA agar plate. The pores permitted the calcium chloride solution to wick down through the filter leaving the spores (15-μm diameter) trapped above. The agar plate was then incubated at 24°C for 24–36 h, during which time the spores germinated and mycelium grew over the surface of the filter; presumably, nutrients diffused through the membrane pores to the growing hyphae. A piece of the filter with mycelium was placed in a small recording chamber and covered with liquid medium. Hyphae continued to elongate throughout this transfer procedure.

Medium Exchange

Medium was exchanged by gravity flow. The recording chamber held 1.5 ml and the maximal exchange rate was 13 ml/min. The time required for complete exchange was measured for potassium; it took 30 s to increase the K^+ concentration by 4 orders of magnitude whereas the equivalent reduction required ~90 s. In many experiments, the rate at which the membrane potential changed was limited by the rate of medium exchange.

Electrical Recording

Microelectrodes were pulled on a Model P-77 Brown-Flaming Micropipette Puller (Sutter Instrument Co.). Relatively rigid electrode tips were required to puncture the cell wall; these microelectrodes typically had resistances of 20–50 MΩ in DMA. Voltage was measured with a Model KS-700 Dual Microprobe System (World Precision Instruments, Inc., New Haven, CT). The bath reference consisted of a DMA agar bridge from the recording chamber to a solution containing 0.5 M potassium acetate and 10 mM KCl; a wire ending in an Ag/AgCl pellet connected this solution to ground.

It was difficult to obtain steady potential recordings for long periods. During most impalements, the measured potential fell off before a reliable value of V_m was recorded. Concomitantly the input resistance increased. I observed microscopically that large vesicles in the cytoplasm migrated toward the puncture site and coalesced around the intruding electrode. They most likely plugged the electrode tip. My attempts to alleviate this difficulty by changing from borosilicate to aluminosilicate glass were unsuccessful. Plugging was also independent of the concentration and type of salt used to fill the micropipettes. However, the addition of a small amount of detergent to the filling solution delayed the sealing process by a few minutes. The best results were obtained with 0.5 M potassium acetate containing 5% Triton X-100; microelectrodes containing this solution often remained unsealed for 5–10 min, and occasionally for as long as 30 min. The detergent did not affect V_m.

Corrections

The recorded V_m had to be corrected by as much as 31 mV due to junction potentials arising at the microelectrode tip. The magnitude of the junction potential was dependent upon the ionic composition of the solution surrounding the electrode tip (2); inserting the electrode into a hypha therefore changed the junction potential and introduced an error into the measurement of V_m. Experiments were designed to estimate the junction potential in each of the media as well as in imitation cytoplasm (31), and these estimates were used to correct the recorded membrane potentials. The magnitude of the junction potential was nearly independent of tip resistance; breaking the tips reduced the potential only slightly. Therefore, a correction value was calculated simply by subtracting the average junction potential in imitation cytoplasm from the average junction potential in the medium; this value was then added to all the membrane potentials recorded in that medium. For example, the average junction potentials in imitation cytoplasm and DMA were +21 and +4 mV, respectively; thus, −17 mV was added to each membrane potential recorded in DMA.

Junction potentials were estimated as follows. First, an electrode filled with 0.5 M potassium acetate was placed in DMA medium and the potential set to zero. Other media were then added to the recording chamber in succession and the change in potential was recorded for each solution and for imitation cytoplasm. This experiment measured the sum of the junction potentials at the electrode tip and at the agar bridge reference. A second experimental procedure was designed to eliminate the potentials arising at the reference. Junction potentials from a 0.5-M potassium acetate–filled electrode were compared to potentials recorded from a 0.5-M KCl-filled electrode; the agar bridge served as reference for both electrodes. Potentials at the two electrodes were set to zero in DMA and then other media were passed through the chamber as before. The difference in the potentials recorded from the two electrodes corresponded to the difference in potentials at their tips. The junction potential at the KCl-filled electrode was expected to be small since K^+ and Cl^- have nearly identical ionic mobilities (40). Potentials recorded with this electrode never varied over 5 mV in any of the media or in imitation cytoplasm. Thus, the differences in the potentials recorded from the two electrodes were almost entirely due to junction potentials at the potassium acetate tip. (The mobilities of K^+ and the acetate anion differ by a factor of two [40].) In all of the experiments described here, V_m was corrected by values ranging from −17 to −31 mV; this adjustment did not change any of the qualitative conclusions.

Results

Hyphae growing in DMA medium maintained a membrane potential of −150 to −170 mV, interior negative. As a starting point, I conducted current–voltage and cable analyses in order to define the electrical properties of the *Achlya* plasma membrane.

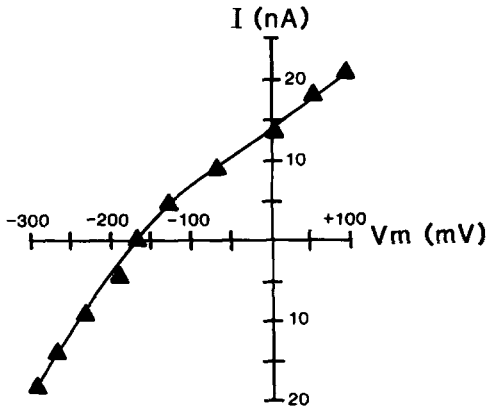

Figure 2. Current–voltage curve for a hypha growing in DMA. Two electrodes were placed ~30 μm apart in a single hypha; short current pulses (500 ms) of increasing intensity were injected with one electrode and the membrane potential was recorded with the other. Alternating pulses of hyperpolarizing current and depolarizing current were injected to prevent electrode polarization.

Current/Voltage Analysis

A current–voltage (I-V) curve describes the response of V_m to current injection; the slope of the curve reveals the relationship between V_m and conductance. The plasma membrane of *Achlya* growing in DMA had a greater conductance at hyperpolarized potentials than at depolarized potentials (Fig. 2). At the resting potential (−167 mV) the input conductance was 1.2×10^{-7} S; it declined to 7.5×10^{-8} S at potentials more positive than −50 mV. Every hypha examined (n = 13) displayed this property; similar results have been reported for *Neurospora* hyphae (9). Although input conductance changed with potential there was no indication of electrical excitability (an abrupt change in conductance), even when V_m was depolarized to +100 mV.

Cable Properties

Linear cable analysis describes the dissipation of a current pulse as it travels along a leaky transmission cable, such as a growing hypha (12). Many of the specific membrane properties of *Achlya* were calculated from this analysis. To simplify matters, a hypha was assumed to be a cable of infinite length whose electrical properties were uniform throughout. Hyphae in DMA medium were impaled with two microelectrodes as far as possible from the nearest tip (>3 mm), making sure that there were no branches between the electrodes. One electrode injected a small current pulse (<1 nA) and the other recorded V_m; the change in V_m was measured at different distances from the current-passing electrode (12). (Unfortunately, the two electrodes could not be inserted more than 2 mm apart due to the proximity of tips and/or branches). Three hyphae were investigated. The space constant (λ), the distance from the current source at which the voltage response falls to e^{-1} of its original value, averaged 2.6 mm. This is comparable to space constants reported for large nerve and muscle fibers (1–3 mm; references 12 and 17) but considerably larger than that of *Neurospora* (500 μm; reference 32). Specific membrane properties calculated from the cable equations varied among the hyphae. Average values were as follows: specific membrane resistance (R_m), 2.4×10^4 ohms·cm^2; specific membrane capacitance (C_m), 1.02 μF/cm^2; and specific internal resistance (R_i), 207 ohm·cm.

In reality, a hypha is not an infinite cable but instead has many lateral branches, each of which terminates in a tip. These geometric complications mean that the values calculated from cable analysis are only approximations. One potential source of error is the relatively short distance between the recording electrode and the nearest tip (≥3 mm). Because this separation is not significantly greater than λ (2.6 mm), the hypha may behave as a closed-end cable in which the injected current pulse rebounds, or reflects, when it reaches the tip. Using a space constant of 2.6 mm, calculations show that this reflecting current could reduce the measured voltage response of the hypha by as much as 10% (12).

The Genesis of the Membrane Potential

In principle, V_m could be generated by passive ionic diffusion, by electrogenic ion pumping, or by both. Diffusion potentials arise from the passive flow of ions down their concentration gradients through membrane channels. Manipulations that alter the concentration gradients across the membrane induce changes in V_m, as described by the Goldman equation. However, V_m of *Achlya* was unaffected by large changes in the concentrations of all the inorganic ions in DMA medium. For example, the K^+ concentration, which was 5.6 mM in normal DMA, was varied from 5 μM to 10 mM without affecting V_m. Similar results were obtained for $HPO_4^=$, Cl^-, Mg^{++}, Na^+, and even Ca^{++}. In fact, simultaneous removal of K^+, Na^+ and Cl^- hyperpolarized V_m by only 10–20 mV. Changes in the pH of the medium had larger effects, but only under extreme conditions (Fig. 3). V_m was hyperpolarized above pH 7.5 and depolarized below pH 5.5; between these pH values it remained at −160 to −170 mV.

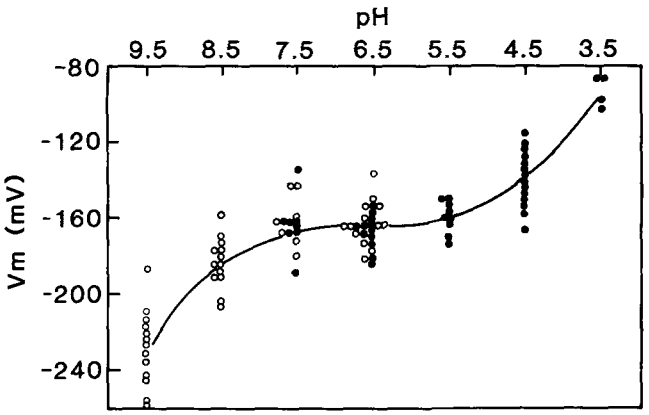

Figure 3. Effect of external pH on V_m. The pH of DMA was adjusted using the following buffers at 1 mM: pH 9.5—CHES (2-[*N*-cyclohexylamino]ethanesulfonic acid); pH 8.5—Tris; pH 7.5—TES (*N*-tris[hydroxymethyl]methyl-2-aminoethane sulfonic acid); pH 6.5—Pipes; pH 5.5—MES (2[*N*-morpholino]ethanesulfonic acid); pH 4.5—acetic acid; and pH 3.5—glycylglycine. In DMA a calcium phosphate precipitate formed at pH 8.5 and pH 9.5. To eliminate precipitation, DMA at high pH was made to contain only 10 μM P_i; as a control, the V_m of hyphae in DMA containing 10 μM P_i was also measured at pH 6.5 and pH 7.5. V_m was not sensitive to the concentration of P_i. (●) DMA containing 1.0 mM P_i; (○) DMA containing 10 μM P_i. Many hyphae were impaled at each pH and each point on the graph represents a single recording. Hyphae were not impaled more than once. Points lying in a row indicate multiple recordings yielding identical values of V_m. Curve drawn by eye.

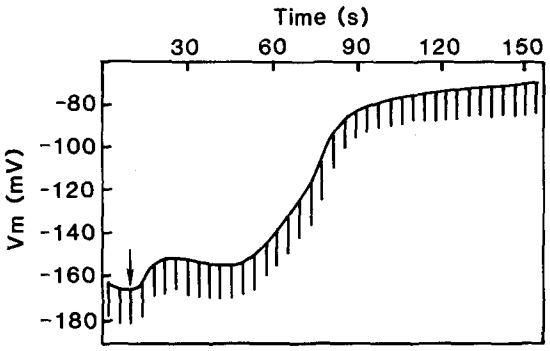

Figure 4. Effect of respiratory inhibition by antimycin A. The trace shows a continuous recording of V_m from a single hypha. At the time indicated by the arrow DMA was exchanged for DMA containing 10 μM antimycin A, which flowed through the chamber for the duration of the time shown. Hyperpolarizing current pulses (1 nA) were injected with the recording electrode throughout the experiment. By dividing the voltage response of the membrane (spikes on recording) by the current intensity, it was possible to calculate the input resistance of the membrane, R_o. In DMA, R_o was 14 MΩ; it fell slightly to 11 MΩ near the end of the depolarization phase and slowly returned to 14 MΩ. The membrane potential of this hypha did not recover over a 15-min period after the antimycin A was removed.

In contrast, V_m was extremely sensitive to respiratory inhibition, suggesting that the plasma membrane contains an electrogenic ion pump. Oxygen consumption by *Achlya* hyphae growing in DMA was abolished within seconds by 8 μM antimycin A; the $Q[O_2]$ fell from 579 to 77 μl/h per mg protein. The response of V_m to 10 μM antimycin A was very similar in all five hyphae examined; a representative recording from one hypha is shown in Fig. 4. Initially, V_m was −165 mV in DMA medium; within 10 s of the time that DMA-containing antimycin A began to flow into the chamber V_m depolarized by 15 mV. The nature of this initial response is unknown but may reflect immediate effects on the plasma membrane. After a delay of 30 s, the potential declined rapidly to −80 mV and then more slowly to −68 mV; the maximal rate of change was 2.6 mV/s. The respiration-independent potential (−68 mV) was not investigated further. The input resistance of the hypha (R_o) was monitored throughout the experiment; R_o remained relatively constant except for a brief period near the end of the depolarization phase when it declined from 14 MΩ to 11 MΩ. Thus, the depolarization was not accompanied by a large shift in the ionic conductance of the plasma membrane. This finding again indicates that V_m is not a diffusion potential because large depolarizations dependent upon diffusion potentials (exemplified by the action potentials of nerve and muscle) characteristically result from a significant increase in membrane conductance.

Effects of Amino Acids on Membrane Potential

The hypothesis that the transcellular current enters hyphal tips by amino acid/proton symport (Fig. 1) implies that amino acid uptake is an electrogenic process. This assumption was tested by investigating the sensitivity of V_m to external amino acids. V_m was recorded ~1 mm behind the tip. (Due to the long space constant [2.6 mm], recordings from this position represent the summation of electrical events occurring over millimeters of hyphal tip.) V_m was hyperpolarized in DMA lacking amino acids; this potential rapidly depolarized when a mixture of the 14 amino acids normally present in DMA was added. In particular, the addition of 1 mM methionine to hyphae bathed in a salts solution at pH 6.5 depolarized V_m by 200 mV (from −320 mV to −120 mV) during the first 20 s (Fig. 5*A*). Over the next 4 min V_m partially repolarized and stabilized at −218 mV. Methionine was then removed by medium exchange; concomitantly V_m hyperpolarized to −329 mV, after which it stabilized at −270 mV. Because methionine is uncharged at pH 6.5, these data indicate that methionine is transported by symport with at least one cation. The gradual readjustment of V_m after depolarization or hyperpolarization is characteristic of symport processes and is thought to reflect compensatory adjustments of other membrane transport sys-

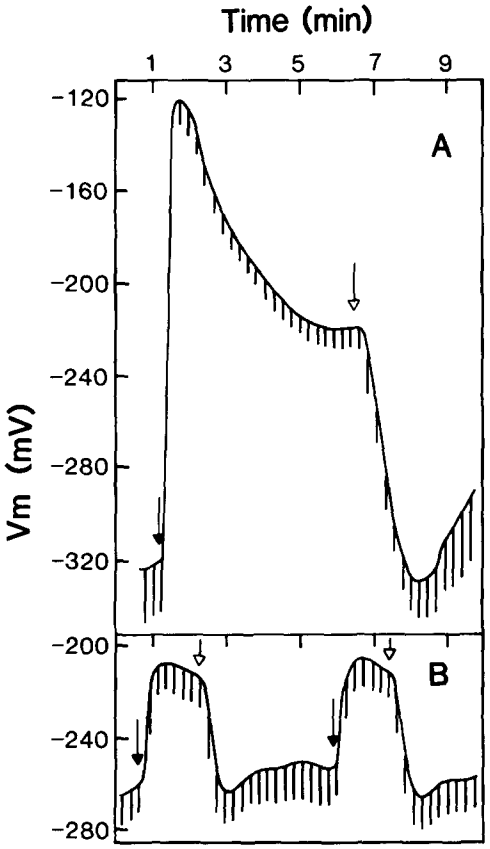

Figure 5. Methionine depolarized V_m. Hyphae on filters were covered with a dilute salts solution lacking methionine; the austerity of this medium caused V_m to be very large. At the times indicated by the filled arrows the same solution plus 1 mM methionine began to flow into the recording chamber. After V_m stabilized the methionine-free salts solution was reintroduced (open arrows). Solution continually flowed through the chamber at a rate of 13 ml/min throughout the experiment. R_o was monitored as described in Fig. 4; 1.5-nA current pulses were injected in *A* and 1-nA pulses in *B*. (*A*) Time course at pH 6.5. The bathing solution initially contained 1 mM calcium hydroxide and 24 mM mannitol for osmotic balance; the pH was adjusted to 6.5 by addition of Pipes (1.3 mM). The rate of change of V_m was limited by the rate of medium exchange, especially during methionine removal. At the start and finish R_o was 16 MΩ; methionine reduced it to 5 MΩ. (*B*) Time course at pH 9.5. A single hypha was subjected to two cycles of methionine addition and removal. The bathing solution initially contained 1.0 mM calcium hydroxide, 1.0 mM CHES, and 24 mM mannitol. The pH was adjusted to 9.5 with Pipes (0.5 mM). V_m was not as large as that in *A*, due to the variability from one hypha to the next. R_o remained constant at 13 MΩ.

tems, specifically ion pumps (10, 18, 28). The input resistance was monitored as described in Fig. 4; R_o reversibly decreased by a factor of 3 during depolarization, from 16 MΩ to 5 MΩ. This decrease was not due to depolarization alone since a depolarization of similar magnitude (induced by current injection through a second intracellular microelectrode) had no effect on R_o (data not shown). Instead, the decrease in R_o was probably due to increased conductance through the methionine symporters.

Both methionine transport and the flow of transcellular current were previously found to be inhibited at high pH (19). Likewise, the depolarization induced by methionine was markedly reduced at pH 9.5 (Fig. 5*B*). Addition of 1 mM methionine reversibly depolarized V_m by only 51 mV and 49 mV in successive trials on a single hypha. R_o was not significantly affected.

In total, seven hyphae were studied at pH 6.5 in either this salt solution or in DMA lacking amino acids; another nine hyphae were examined in the same two media at pH 9.5. Depolarizations induced by methionine were three- to fivefold larger at pH 6.5 than at pH 9.5. In DMA minus amino acids, 1 mM methionine depolarized V_m by 86.3 ± 14.5 mV (mean ± SEM) at pH 6.5 but only 17.8 ± 6.3 mV at pH 9.5. In the salt solution the values were 160.5 ± 12.4 mV at pH 6.5 and 48.6 ± 3.2 mV at pH 9.5.

Intracellular Voltage Gradient

Current flow through the apical cytoplasm may be expected to generate an internal electric field. The magnitude of this field was measured directly by recording V_m at increasing distances behind the tip. In every hypha examined (n = 15), V_m near the tip was at least 10 mV more positive than the potential recorded 500 μm back; three examples are shown in Fig. 6. In most cases the change in V_m was greatest over the first 200 μm, and the potential reached a plateau value 500 to 1,000 μm behind the tip. For practical purposes, the extracellular medium was at constant potential so that the differences in V_m must be due to a potential gradient across the cytoplasm, tip positive. The field strength was estimated conservatively at 10 mV/500 μm, or 0.2 V/cm. The data from all 15 hyphae were normalized and plotted on a single curve (inset to Fig. 6). Membrane potentials recorded at all positions within 400 μm of the tip were depolarized with respect to those recorded greater than 500 μm back, but the extent of depolarization at each position varied considerably as indicated by the error bars. This variability may be a result of differences in the intensity of the cytoplasmic current density among the hyphae. On average, the cytoplasm 50 μm behind the tip was 20.6 ± 6.7 mV electropositive, corresponding to a field of 0.4 V/cm.

No significant internal voltage gradient was detectable under conditions that inhibited the flow of transcellular current; removal of amino acids or increasing external pH had this effect (19). For these experiments hyphae were grown on filters so that the medium could be exchanged; unfortunately it proved difficult to impale them repeatedly without damaging the cells. Nonetheless large, stable potentials were recorded from some hyphae. In DMA lacking amino acids V_m near the tip was never more than 3 mV more positive than potentials recorded farther back (n = 10); a representative potential profile from one of these hyphae is shown in Fig. 7*A*. In four of the hyphae, the apical potential was slightly hyperpolarized with respect to more distal potentials. When the recordings from all 10 hyphae were normalized and plotted together, the hyphal cytoplasm appeared to be nearly isopotential (Fig. 7*A*, inset). At elevated pH (9.5), hyphae were extremely sensitive

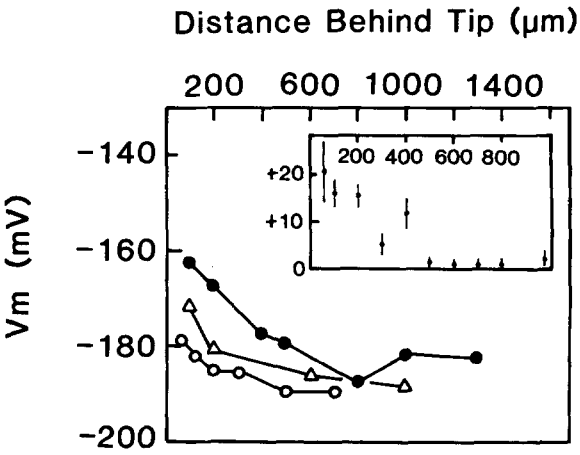

Figure 6. The gradient of membrane potential. (*A*) Representative data from three hyphae growing in DMA at pH 6.5. Mycelium was grown directly on the surface of DMA agar; the agar provided physical support which facilitated multiple impalements of a single hypha. The V_m of hyphae on filters in liquid DMA was also depolarized by at least 10 mV near the apex. For unknown reasons, V_m of hyphae grown directly on DMA agar was always 10–20 mV more negative than that recorded from hyphae on filters. Inset shows the pooled data from all 15 hyphae after normalization. The potentials from each hypha were normalized by assigning the value of OmV to the most negative V_m recorded along that hypha; potentials at all other positions thereby became ≥OmV. Points represent the mean of the normalized potentials at each position and bars represent the SEM.

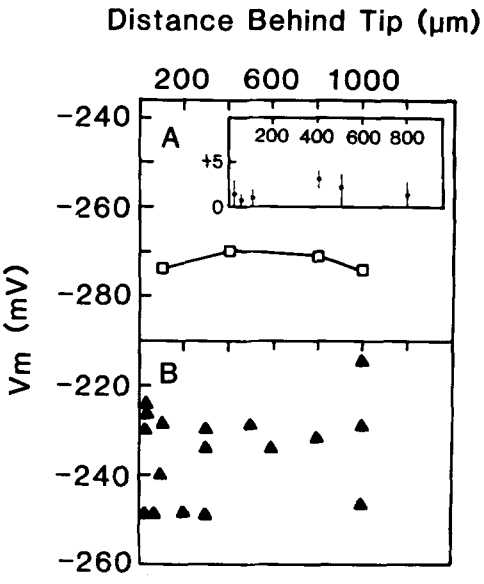

Figure 7. Intracellular potential profile after current flow was inhibited. (*A*) Representative potential profile from a hypha in amino acid–free DMA. Hyphae were grown on filters so that the medium could be exchanged. Inset shows the pooled data from 10 hyphae in amino acid–free DMA after the measurements were normalized as in Fig. 6. (*B*) Potentials recorded from hyphae in DMA buffered to pH 9.5 with 1.0 mM CHES. Each point represents a measurement from a separate hypha.

to damage and the apical cytoplasm was filled with vesicles that sealed the electrode tip almost instantaneously. For these reasons, it was not possible to obtain multiple recordings from an individual hypha, so potential profiles like those shown in Figs. 6 and 7*A* could not be constructed. Instead single recordings from many hyphae were pooled (Fig. 7*B*). Nevertheless, the result with elevated pH was the same as that in amino acid–free DMA: no clear difference could be detected between the potentials at the tip and those at the trunk.

Discussion

Internal Voltage Gradient

The data presented here demonstrate that *Achlya* hyphae possess an internal voltage gradient associated with the transhyphal current flow. The field strength calculated from this voltage gradient measures 0.2 V/cm. Conditions that inhibited circulation of the proton current also abolished the voltage gradient; reducing the concentration of amino acids or protons in the medium stopped the current, hyperpolarized V_m, and caused the cytoplasm to become isopotential. This finding suggests that the measured potential gradient was not an artifact created by membrane damage during impalement. If the depolarized potentials recorded in apical regions were simply the result of tips being more easily damaged than trunks, one would have expected the potential gradient to remain, and perhaps to intensify, when the membrane potential was hyperpolarized.

The link between the field and the proton current is strengthened by their spatial distributions; both are restricted to the first millimeter of hyphal length, with peak intensities in the terminal 200 μm. Moreover the polarity of the field is consistent with the orientation of current flow.

In principle, internal current flow must generate a cytoplasmic electric field; the measured field intensity therefore was compared to that predicted from theoretical considerations. Using Ohm's law, the field strength is the product of the cytoplasmic current density and the specific internal resistance. I estimate the current density in *Achlya* hyphae to be 150 $\mu A/cm^2$, assuming that all of the current which enters the tip flows distally through a uniformly conductive cytoplasm. This calculation was based on the profile of current strength shown in Fig. 3*C* of reference 11 with the maximal intensity of inward current equal to 2.5 $\mu A/cm^2$. (In a previous publication [20] we underestimated the internal current density.) R_i, as calculated from cable analysis, is 200 ohm·cm. The predicted internal field strength is then 0.03 V/cm, nearly an order of magnitude less than that measured.

By contrast, L. F. Jaffe et al. have suggested that transcellular currents carried by calcium ions or protons may create internal fields that are as much as three orders of magnitude greater than that predicted from Ohm's law (16). Their calculations rest upon the assumption that these two ions are not free to carry current through the cell. Instead, they postulate that Ca^{++} and H^+ are bound in the cytoplasm at the site of current entry, thus creating very large, internal fixed-charge gradients. It is possible that this mechanism contributes to the field measured in *Achlya*.

For many cell types that drive ionic currents, such calculations are the only means to estimate the cytoplasmic field strength. Direct measurement is impractical. Repeatedly impaling small cells such as *Pelvetia* embryos, *Fucus* embryos, or plant root hairs certainly would damage them. In addition, the voltage gradient across these cells is likely to be quite small, and perhaps below the limit of detection, owing to their short lengths.

For these reasons, *Achlya* is only the third organism in which single cells have been shown to possess both a transcellular current and an internal voltage gradient; *Neurospora* and *Amoeba* being the other two. In 1962, Slayman and Slayman reported that the membrane potential near the tip of *Neurospora* hyphae is 100 mV more positive than the potential recorded one centimeter back, and most of the voltage drop is restricted to the first millimeter (33). More recently, Gow demonstrated that these hyphae drive a transhyphal current that enters the tip (7). In *Amoeba* the V_m of an advancing pseudopod is depolarized with respect to that of the trailing cell body (4), and extracellular current flows into the pseudopod (24). Unfortunately, in neither organism has it been shown that the internal voltage gradient is coupled to the extracellular current.

Voltage gradients and currents detected in follicles of the *Cecropia* moth also deserve attention. In this system, the oocyte is connected to its seven nurse cells by cytoplasmic bridges. Although the internal electrical phenomena are not truly intracellular, they still may be cytoplasmic in nature. Woodruff and Telfer provided convincing evidence that the oocyte is 10 mV electropositive with respect to the nurse cells (43), and it was later shown that the current pattern surrounding the follicle, although somewhat complex, is consistent with the orientation of the internal field (15).

Does the endogenous cytoplasmic field serve a physiological purpose? This question lies at the heart of research in this area, but as yet there is no clear answer. It is attractive to speculate that in *Achlya* the field plays a role in the localization of growth, since the anode (positive pole) resides in the cytoplasm at the elongating apex. Theoretically, the magnitude of the field is sufficient to redistribute charged proteins and organelles within the hypha, a process termed self-electrophoresis (13, 14, 16). Most proteins and organelles bear a net negative charge on their surface and so would be subject to an electrophoretic force directed toward the tip. The mobilities of highly charged proteins, such as prealbumin (0.8 μm/s per V per cm [36]), and most organelles (~4 μm/s per V per cm [13, 27, 37]) are such that the measured field of 0.2 V/cm would transport these constituents at rates of 10 μm/min or greater, ignoring back-diffusion. Since the hyphal tip elongates at only 5 μm/min, these cellular constituents may be localized in the apical cytoplasm by the electric field.

We previously discounted the self-electrophoresis mechanism (20) based on two lines of evidence: (*a*) calculations using Ohm's law predicted the internal field to be too small to localize material at the elongating apex; and (*b*) the emergence of a new branch sometimes caused the direction of current flow at the pre-existing tip to reverse for up to 40 min without affecting the rate of tip elongation. This conclusion must be reconsidered in light of the evidence presented here. The measured field is indeed large enough to affect cytoplasmic localization. This places increased significance on the behavior of the field during current reversal, which has not yet been determined. The technique of intracellular recording is unlikely to be of use in such a study owing to the rarity of

current reversal (only documented in nine branching hyphae), the short duration of reversal (40 min or less), and the difficulty in obtaining stable potential recordings in *Achlya*. For the present, the cytoplasmic field must be considered a possible mechanism of hyphal polarization.

The Genesis of V_m

It is now well established that an electrogenic proton pump governs V_m in *Neurospora*. In a series of papers, Slayman and his co-workers demonstrated that V_m was critically dependent on the cellular ATP level which, in turn, was supported by respiration and oxidative phosphorylation (32, 34, 35). They concluded that the control of V_m was dominated by an electrogenic ion pump. Using membrane vesicles, Scarborough demonstrated that the pump was a plasma membrane ATPase (29) that ejected protons (30).

The case for *Achlya* is less complete, but the potential appears to arise in the same way. Diffusion potentials do not make a significant contribution to V_m, as judged by ion exchange experiments. Instead, the large negative values of V_m, sometimes exceeding −300 mV, are characteristic of electrogenic ion pumping. Respiration clearly supports this potential gradient, probably by energizing the synthesis of ATP. The sensitivity of V_m to pH extremes may indicate that the putative pump expels protons; such H^+-ATPases have been identified in the plasma membranes of yeast (6), *Neurospora* (see above), and higher plants (25, 39).

Might the pump that generates V_m also be the source of the outward limb of the transhyphal proton current (depicted in Fig. 1)? We previously speculated that an electrogenic H^+-ATPase generated the outward current (19). The data presented here are consistent with this proposal insofar as they suggest an electrogenic pump in the plasma membrane. It is noteworthy that respiratory inhibition blocks both the pump and the transhyphal proton current. However, the identity of the ion(s) ejected by the ATPase remains uncertain.

Electrogenic Amino Acid Transport

In bacteria, plants, and fungi, the uptake of amino acids is driven by the electrochemical gradient of protons (5, 11, 26) as originally formulated by Peter Mitchell in the chemiosmotic theory (21). We postulated that such a membrane transport system could generate the inward current at the hyphal tips if the transport were electrogenic and if the symporters were localized in the apical membrane. Although the physical distribution of the amino acid transport systems has not been investigated, the transport process is clearly electrogenic. Attention was focused on methionine transport because this amino acid supports most of the inward current (19). The addition of 1 mM methionine rapidly and reversibly depolarized V_m by 200 mV. Both the depolarization and methionine uptake (19) were inhibited at pH 9.5, suggesting that protons carry the charge into the hypha during methionine transport. These findings are consistent with the proposal that localized amino acid/proton symport carries inward current.

I thank Drs. F. M. Harold and J. H. Caldwell for invaluable guidance and stimulating discussion. Diane Nicholl provided technical assistance and Karen Bruder prepared the manuscript; I thank both of them.

This research was supported by National Institutes of Health grants AI03568 and NS16922, as well as National Science Foundation grant PCM8009439.

Received for publication 5 February 1985, and in revised form 11 November 1985.

References

1. Armbruster, B. L., and M. H. Weisenseel. 1983. Ionic currents traverse growing hyphae and sporangia of the mycelial water mold *Achlya debaryana*. *Protoplasma*. 115:65–69.
2. Barry, P. H., and J. M. Diamond. 1970. Junction potentials, electrode standard potentials, and other problems in interpreting electrical properties of membranes. *J. Membrane Biol.* 3:93–122.
3. Behrens, H. M., M. H. Weisenseel, and A. Sievers. 1982. Rapid changes in the pattern of electric current around the root tip of *Lepidium sativum* L. following gravistimulation. *Plant Physiol. (Bethesda).* 70:1079–1083.
4. Bingley, M. S. 1966. Further investigations into membrane potentials in amoebae. *Exp. Cell Res.* 43:1–12.
5. Eddy, A. A. 1982. Mechanisms of solute transport in selected eukaryotic microorganisms. *Adv. Microb. Physiol.* 23:1–78.
6. Goffeau, A., and C. W. Slayman. 1981. The proton translocating ATPase of the fungal plasma membrane. *Biochim. Biophys. Acta.* 639:197–223.
7. Gow, N. A. R. 1984. Transhyphal electrical currents in fungi. *J. Gen. Microbiol.* 130:3313–3318.
8. Gow, N. A. R., D. L. Kropf, and F. M. Harold. 1984. Growing hyphae of *Achlya bisexualis* generate a longitudinal pH gradient in the surrounding medium. *J. Gen. Microbiol.* 130:2967–2974.
9. Gradmann, D., U.-P. Hansen, W. S. Long, C. L. Slayman, and J. Warncke. 1978. Current-voltage relationships for the plasma membrane and its principle electrogenic pump in *Neurospora crassa*. I. Steady-state conditions. *J. Membr. Biol.* 39:333–367.
10. Gunter-Smith, P. J., E. Grasset, and S. G. Schultz. 1982. Sodium-coupled amino acid and sugar transport by *Necturus* small intestine. An equivalent electrical circuit analysis of a rheogenic co-transport system. *J. Membr. Biol.* 66:25–39.
11. Harold, F. M. 1977. Ion currents and physiological functions in microorganisms. *Annu. Rev. Microbiol.* 31:181–203.
12. Jack, J. J. B., D. Noble, and R. W. Tsien. 1975. Electric Current Flow in Excitable Cells. Clarendon Press, Oxford. 25–82.
13. Jaffe, L. F. 1969. On the centripetal course of development, the *Fucus* egg, and self-electrophoresis. *Dev. Biol. Suppl.* 3:83–111.
14. Jaffe, L. F., and R. Nuccitelli. 1977. Electrical controls of development. *Annu. Rev. Biophys. Bioeng.* 6:445–476.
15. Jaffe, L. F., and R. I. Woodruff. 1979. Large electrical currents traverse developing *Cecropia* follicles. *Proc. Natl. Acad. Sci. USA.* 76:1328–1332.
16. Jaffe, L. F., K. R. Robinson, and R. Nuccitelli. 1974. Local cation entry and self-electrophoresis as an intracellular localization mechanism. *Ann. NY Acad. Sci.* 238:372–389.
17. Katz, B. 1966. Nerve, Muscle, and Synapse. G. Wald, editor. McGraw-Hill Book Co., New York. p. 75.
18. Kinraide, T. B., and B. Etherton. 1982. Energy coupling in H^+-amino acid cotransport. ATP dependence of the spontaneous electrical repolarization of the cell membranes in oat coleoptiles. *Plant Physiol. (Bethesda).* 69:648–652.
19. Kropf, D. L., J. H. Caldwell, N. A. R. Gow, and F. M. Harold. 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.
20. Kropf, D. L., M. D. A. Lupa, J. H. Caldwell, and F. M. Harold. 1983. Cell polarity: endogenous ion currents precede and predict branching in the water mold *Achlya*. *Science (Wash. DC).* 220:1385–1387.
21. Mitchell, P. 1966. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biol. Rev. Camb. Philos. Soc.* 41:445–502.
22. Nuccitelli, R. 1978. Ooplasmic segregation and secretion in the *Pelvetia* egg is accompanied by a membrane-generated electrical current. *Dev. Biol.* 62:13–33.
23. Nuccitelli, R., and L. F. Jaffe. 1975. The pulse current pattern generated by developing fucoid eggs. *J. Cell Biol.* 64:636–643.
24. Nuccitelli, R., M.-M. Poo, and L. F. Jaffe. 1977. Relations between ameboid movement and membrane-controlled electrical currents. *J. Gen. Physiol.* 69:743–763.
25. O'Neill, S. D., and R. M. Spanswick. 1984. Solubilization and reconstitution of a vanadate-sensitive H^+-ATPase from the plasma membrane of *Beta vulgaris*. *J. Membr. Biol.* 79:231–243.
26. Poole, R. J. 1978. Energy coupling for membrane transport. *Annu. Rev. Plant. Physiol.* 29:437–460.
27. Sack, F. D., D. A. Priestley, and A. C. Leopold. 1983. Surface charge on isolated maize-coleoptile amyloplasts. *Planta (Berl.).* 157:511–517.
28. Sanders, D., C. L. Slayman, and M. L. Pall. 1983. Stoichiometry of H^+/amino acid cotransport in *Neurospora crassa* revealed by current-voltage analysis. *Biochim. Biophys. Acta.* 735:67–76.
29. Scarborough, G. A. 1976. The *Neurospora* plasma membrane ATPase is an electrogenic pump. *Proc. Natl. Acad. Sci. USA.* 73:1485–1488.
30. Scarborough, G. A. 1980. Proton translocation catalyzed by the electrogenic ATPase in the plasma membrane of *Neurospora*. *Biochemistry.* 19:2925–2931.

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

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

33. Slayman, C. L., and C. W. Slayman. 1962. Measurement of membrane potentials in *Neurospora*. *Science (Wash. DC)*. 136:876–877.

34. Slayman, C. L., W. S. Long, and C. Y.-H. Lu. 1973. The relationship between ATP and an electrogenic pump in the plasma membrane of *Neurospora crassa*. *J. Membr. Biol.* 14:305–338.

35. Slayman, C. L., C. Y.-H. Lu, and L. Shane. 1970. Correlated changes in membrane potential and ATP concentrations in *Neurospora*. *Nature (Lond.)*. 226:274–276.

36. Sober, H. A. (editor). *In* Handbook of Biochemistry, second edition. 1970. CRC Press, Cleveland. p. C36.

37. Stocking, C. R., and V. R. Franchesi. 1982. Some properties of the chloroplast envelope as revealed by electrophoretic studies of intact chloroplasts. *Plant Physiol. (Bethesda)*. 70:1255–1259.

38. Stump, R. F., K. R. Robinson, R. L. Harold, and F. M. Harold. 1980. Endogenous electrical currents in the water mold *Blastocladiella emersonii* during growth and sporulation. *Proc. Natl. Acad. Sci. USA*. 77:6673–6677.

39. Vara, F., and R. Serrano. 1982. Partial purification of the proton translocating ATPase of plant plasma membranes. *J. Biol. Chem.* 257:12826–12830.

40. Weast, R. C. (editor). *In* Handbook of Chemistry and Physics, 65th edition. 1985. CRC Press, Inc., Boca Raton, Florida. p. D171–D172.

41. Weisenseel, M. H., A. Dorn, and L. F. Jaffe. 1979. Natural H^+ currents traverse growing roots and root hairs of barley (*Hordeum vulgare* L.). *Plant Physiol. (Bethesda)*. 64:512–518.

42. Weisenseel, M. H., R. Nuccitelli, and L. F. Jaffe. 1975. Large electrical currents traverse growing pollen tubes. *J. Cell Biol.* 66:556–567.

43. Woodruff, R. I., and W. H. Telfer. 1973. Polarized intercellular bridges in ovarian follicles of the cecropia moth. *J. Cell Biol.* 58:172–188.