Modifications of Extracellular Electric and Ionic Gradients Preceding the Transition from Tip Growth to Isodiometric Expansion in the Apical Cell of the Fern Gametophyte

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ABSTRACT

Fern (Onoclea sensibilis L.) gametophytes exposed to blue light are induced to undergo a morphological transition from a tip-growing filament to a planar prothallus. Extracellular measurements of electric currents and localized ion activities around the apical cell of 8 to 10 day-old gametophytes were made with a vibrating probe and ion selective electrodes. In darkness, we observed exit current densities of an average of 75 nanoamperes per square centimeter near the tip and 2 to 15 nanoamperes per square centimeter along the lateral walls of this cell. Measurements with ion selective electrodes for H+, K+, and Ca2+ showed that this cell was bounded by a thin layer of medium that was depleted in K+ and Ca2+ and exhibited a lower pH than the bulk solution. Both the K+ and Ca2+ depletion zones and the zone of higher acidity were particularly pronounced at the tip end of the cell; the pH at 2 micrometers from the tip was nearly 0.5 units more acid than the bulk medium at pH 6. Disruption of steady state, external gradients with media that contained lower concentrations of H+, K+, Ca2+, or CI- produced certain differences in the rates of restoration of particular ion zones, raising the possibility that some of the ion migrations are interdependent. Within 15 minutes after irradiation with blue light, current leaving the tip declined to levels which were indistinguishable from those leaving the lateral walls and there was a rapid lowering in the rates of tip acidification and K+ depletion near the tip. The rapid dissipation of both the longitudinally aligned electrical field and the tip-localized asymmetries in external cation distribution in blue light suggest that loss of electrical polarity in this tip growing cell may be an initial step in the chain of events which govern changes in cell shape.

The induction of cellular polarity is often observed as the first event in the morphogenesis of single-celled plants. Recent investigations on how apolar plant structures establish polarized growth have strongly implicated endogenous ionic currents as the causal agent in axis establishment (2, 14, 31). Electric fields have been shown to anticipate and/or accompany polarized growth in single-celled structures as diverse as algal zygotes (18, 19), barley root hairs (32), lily pollen tubes (30, 33), and fungal hyphae (1, 12, 16). These tip-growing structures produce steady, self-generated ionic currents in the range of 1 to 5 μamp cm−2 that traverse the length of the structure.

We have been studying the process of cellular polarity in the gametophyte generation of the fern Onoclea sensibilis L. The filamentous portion of this organism is an intriguing experimental system to investigate the role of localized ionic currents in cellular polarity because it differs from the other tip-growing systems studied to date in several important features. Unlike the other structures that are specialized for nutrient uptake, the fern filament is specifically designed to carry out photosynthesis; any electric fields measured around the filament are more likely to have developmental, instead of nutritional significance. Second, the ability of blue light to alter the growth pattern from the tip-growing filament to the two-dimensional prothallus (8, 11) provides the rather unique opportunity to determine if a change in field orientation consistently precedes the change in growth polarity.

Our studies with conventional microelectrodes showed that the apical cell of dark grown filaments acts as a cellular dipole: the tip is 5 mV electronegative relative to the base (22). Irradiation with 10 to 120 s of blue light promoted further hyperpolarization of the tip by 5 mV over a 3 min period; however, a separate microelectrode located at the base of the cell showed little change in the membrane potential in this region. About 5 min after the tip had hyperpolarized, the microelectrode in the base revealed a similar, but larger (15 mV), hyperpolarization of the membrane at the base, even though the organism had remained in darkness following its initial exposure to light. Together, these events make the cell interior equipotential within 10 min after the onset of blue light (22). Inhibitor studies have suggested that the photoreceptor which mediates the initial electrical events as well as the ultimate morphological response is a flavoprotein coupled to an electron transport chain in the plasma membrane (5). Regardless of whether the activity of the electron transport chain itself contributes to membrane hyperpolarization, the sensitivity of both electrical and morphological response to vanadate (5) implies that the activated photoreceptor is also able to stimulate H+-pumping ATPases of the apical cell plasma membrane.

In this study, we present additional observations of the electrical and ionic events in the fern gametophyte apical cell during the light triggered transition from tip growth to isodiometric expansion in blue light. Our primary objectives were to determine whether the dark-grown filament generated a cellular field similar to those seen in other tip-growing cells and to identify the ions that carry this current. Second, in order to gain further understanding of how blue light regulates gametophyte morphogenesis, we monitored changes in media-borne currents and in the free concentration of ions following blue light irradiation. (Note: We use the term 'concentration' as a general term to define the molar composition of bathing media. Since the ion selective electrodes actually monitor ion activity, rather than concentration, we have adopted the use of the term 'free ion concentration' to make clear the distinction between the concentration of fully ionized cation and anion species in solution and the concen-

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trations of those ions which are nonionized or bound.)

**MATERIALS AND METHODS**

**Culture Conditions.** The culture conditions followed those described by Cooke and Racusen (3) for the preparation of *Onoclea sensibilis* L. spores. Briefly, spores obtained from Tompkins County, NY, were wetted with a 0.1% Triton X-100 detergent solution and then sterilized with a 1% Clorox wash for 75 s. These spores were rinsed three times in sterile deionized water and plated on artificial pond water media (0.5 mm NaCl, 0.1 mm KCl, and 0.1 mm CaCl₂) supplemented with 1% sucrose, 0.8% agar, and buffered with 1 mm 2-morpholinoethanesulfonic acid (MES, pH 6). This medium, with various adjustments made in H⁺, K⁺, or Ca²⁺ concentrations, was also used in experiments, but contained no agar or buffer in those applications. All media solutions used for bathing gametophytes and calibrating ion sensitive electrodes (see below) were made with deionized water which had a measured resistivity in excess of 15 megohm-cm, and were prepared and stored in polyethylene labware. Game-

...phophytes were rinsed with 0.52 mM CaCl₂ (WP Instruments) and dried on the tip of a sterile pipet. Ager was added to the bath solution as necessary.

Prepared spores were germinated under cool-white fluorescent lights with an intensity of 5 W/m² for 24 h and then stored in the dark for 8 to 10 d.

**Ion-Selective Electrodes.** Borosilicate glass capillaries with an interior filament (Kwik-Fil Capillaries, WP Instruments, New Haven, CT) were soaked for 1 h in a potassium dichromate-sulfuric acid solution, rinsed in deionized water followed by acetone, and dried at 110°C. Washed capillaries were pulled to a tip diameter of approximately 1 μm. The tip interior was silanized by dipping the tip in a 5% silane (1,1,1,3,3-hexamethyldisilazane, Aldrich Chemical Co, Milwaukee, WI) solution in xylene for 1 min and then baking the electrode for 45 min at 80°C. Silanized microelectrodes were dipped into the exchange resin (WP Instruments) to allow the distal 300 μm of the tip to fill, and were then back-filled with the appropriate salt solution (H⁺: 0.04 M K₂HPO₄, 0.023 M NaOH, 0.015 M NaCl [pH 7.0]; K⁺: 0.5 M KCl [pH 7.0]; Ca²⁺: 0.1 M CaCl₂ [pH 7.0]). Finally, the entire electrode barrel, except for the very tip and the area in contact with the holder, was coated with a silver-based epoxy (E-solder No. 3021, Acme Chem., New Haven, CT). Typical resistances for hydrogen ion electrodes were 10¹⁰ ohm (Ω), for potassium electrodes 10⁹ Ω, and calcium electrodes 10¹¹ Ω. Reference electrodes were formed by coupling a Ag/AgCl wire to the bathing solution via a salt bridge consisting of a 2.5% agar mixture of the appropriate back-filling solution.

Each type of ion selective electrode was calibrated in solutions with known concentrations of the relevant cation. The calibration solutions for H⁺ consisted of APW media made to cover 4 decades of pH from 8 to 4. The H⁺ sensitive electrodes came the closest to ideal Nerstian behavior, with 57 to 60 mV responses/decade concentration for the entire range tested. The K⁺ and Ca²⁺ electrodes were similarly calibrated in APW media in which the ions were supplied to cover a 3 decade range from 10⁻⁴ to 10⁻¹⁰ M. The K⁺ electrodes showed fairly linear responses through the calibration series with 52 to 57 mV/decade changes being typical. The Ca²⁺ electrodes exhibited 32 to 36 mV/decade responses in the range of 10⁻⁴ to 10⁻¹⁰ M and 27 to 33 mV/decade response when Ca²⁺ was lowered to 10⁻⁷ M. To ensure that calibration solutions containing very low levels of Ca²⁺ were not contaminated with Ca²⁺ from the deionized water or the chemicals used to prepare APW media, we performed several calibrations of Ca²⁺ sensitive electrodes in EGTA buffered APW media using formulas described by Tsien and Rink (28). We observed no marked differences in the Ca²⁺ electrode electrical responses to decade shifts in the Ca²⁺ concentration of solutions made with or without EGTA buffering. This finding is significant for experiments in which we wished to monitor Ca²⁺ uptake into gametophytes because we have observed that tip growth in gametophytes is inhibited in the presence of even low levels of EGTA (TJ Cooke, unpublished observations), and because the presence of a Ca²⁺ buffering agent would preclude the possibility of observing changes in external Ca²⁺ during medium exchange experiments.

Small agar blocks with surface-adhering gametophytes were excised from the culture plates and placed against the back wall of a plexiglass chamber. The gametophytes were viewed through a horizontally positioned microscope which oriented the experimental chamber in a vertical position against the stage. Chamber dimensions were roughly 1 cm high × 2 cm long × 0.3 cm thick. When desired, solutions could be changed with a flow-through system consisting of tubes and reservoirs connected to the opposite sides of the chamber. Alterations in ion composition of the media were accomplished by modifying the concentrations of the salts which made up APW medium. A constant level of total ion activity was maintained by adding appropriate amounts of NaCl or Na₂SO₄ to the medium. Ion selective electrodes were positioned outside the cell at various distances from the tip or determined walls of the apical cell. Changes in ion activities were monitored with a high impedance (10¹⁰ Ω) electrometer (FD-223 Dual/Differential Electrometer, WP Instruments, New Haven, CT) which was connected to a chart recorder. Light conditions were controlled by a series of interference and absorption filters on the microscope lamp which provided narrow band green (peak wavelength = 548 nm) or blue (peak wavelength = 454 nm) light (22). Experiments utilizing ion selective electrodes are essentially limited to two types of data collection: either one measures the activity at the point of interest and compares it to a like measurement taken at some reference point (mapping), or one measures changes in local activity following experimental disruption of the steady state (fluxes). In mapping experiments we measured ion activities at distances ranging from 2 to 20 μm of the tip and/or base of the cell with respect to a reference position 200 μm from the cell. This was done by frequent alternation of electrode position between measuring sites proximal and distal to the cell. For purposes of evaluating trends in rates of influx or efflux, the steady state conditions for external ion activities were altered by flushing through three chamber volumes (approximately 1.5 ml total volume) of the desired APW-based medium in 1 min. Electrodes for H⁺, K⁺, or Ca²⁺ were immediately placed at the selected measuring site and changes resulting from this dissipation of localized ion accumulation or depletion zones were recorded. It should be noted that the dissipation of the preexisting ion distributions near the cell following infusion of new medium required that the rate of solution movement be sufficient to reduce the unstirred layer of medium that exists under static (no flow) conditions. We estimate that the unstirred layer of approximately 20 μm in static conditions (based on steady state H⁺ accumulation near the tip, see Fig. 3) is reduced to 1 μm or less during medium exchange experiments. This reduction was achieved by repeated experiments in which we were able, with comparable flow rates, to wash away 300 to 500 nm ion exchange resin beads that were adjacent, but unattached, to the cell wall. In those cases where the measured activity of a particular ion began to rise, we concluded that the cell was extruding ions to return to steady state. Likewise, decreases in external ion activity were interpreted as ion influx. Obviously, no change in activity indicated either that there were no localized differences in activity for that ion, or that such differences existed but were not accessible to the infusion of fresh medium.

**Vibrating Probe.** A vibrating probe system, similar to that described by Jaffe and Nuccitelli (13) during the introduction of this technique, was used to measure currents around fern gametophytes. Probes were constructed from gold plated, glass capillary pipets filled with melted and resolidified solder as de-
scribed in the original paper. The electroplated platinum ball on the tip of the probe, which serves as the voltage-sensing portion of the instrument, was 10 to 15 µm in diameter. Probes were bonded to a lucite holder (similar to the 'boat' described in the original paper) with silicone cement and this assembly was attached to the piezo electric 'bender' element on the micromanipulator arm. Probes were calibrated and adjusted following procedures outlined in Jaffe and Nuccitelli (13) using known currents injected from a glass micropipet into APW medium.

Current is defined in our system in the conventional sense, that is, as the flow of positively charged particles. For calibration procedures and during experiments the probe was vibrated at 150 to 300 Hz, with an excursion distance of 10 to 20 µm. With a lock-in amplifier time constant of 10 s and a medium resistivity of 10^4 Ω·cm these vibration parameters were expected to have a noise threshold of about 6 nA/cm².

Gametophytes grown on agar were transferred under dim green safelights to a horizontally mounted perfusion chamber by excising a section of agar from the dish. This chamber had a fluid volume of about 1.5 ml and was equipped with small-diameter tubing to allow slow perfusion (0.1 ml/min) of the dish with fresh APW medium. This flow rate was increased to 2 ml/min for a period of 2 min for experiments in which the steady state distribution of ions around the gametophyte was to be altered. Medium conductivity was measured before and after infusion of a medium with different ionic composition. The perfusion chamber was attached to a specially designed holder on the X-Y stage positioner of a Zeiss model D inverted microscope. Microscopic observation of the gametophyte during probe measurements was done in green light provided by filtering the 60 W tungsten light source through a sandwich of a Zeiss interference filter (peak wavelength 546, bandwidth 10 nm) and two layers of a broadband absorption filter (Roscolene 871, Roscoe Labs, Port Chester, NY). Further adjustments of light intensity were obtained by positioning glass neutral density filters in front of the green filter pack. In certain experiments gametophytes were exposed to blue light by irradiating a small region of the dish with a fiber optic light source positioned about 5 mm from the preparation. The fiber optic transmitted blue light from a filtered tungsten source (peak wavelength 454 nm, bandwidth 10 nm) to the dish with a beam diameter at the surface of the agar plate of about 1 mm and an intensity of 0.2 W/m². It was previously determined that the blue light from this source was sufficient for eliciting isodiametric expansion in the apical cells of over 90% of gametophytes tested.

Because our experiments required making field measurements on several gametophytes before and during blue light irradiation, we devised a system of gametophyte mapping coordinates so that current measurements at various locations around the gametophyte apical cell were somewhat standardized and, thus, could be directly compared. These coordinates consisted of an inner and outer shell of measuring sites (Fig. 6), each of which were manually registered by noting the x-y coordinates on the calibrated stage positioner. In this way it was possible to change position of the perfusion chamber during measurements and be able to relocate any of a number of selected gametophytes from which earlier measurements were made. At every mapping site, for each selected gametophyte, we made two determinations of endogenous currents; one was made with the probe vibrating normal to the longitudinal axis of the gametophyte, the other with the probe vibrating parallel to the growth axis. These orthogonal components permitted us to deduce a single vector which reflected the relative size and direction of medium-borne currents.

**Data Acquisition and Analysis.** Electrical signals from both the vibrating probe and ion selective electrodes were recorded on Houston instruments model 1000 strip chart recorders. For some of the figures in the text it was possible to reproduce the original traces from these chart records. In most cases the analog records were converted into digital form by tracing over the original records on a digitizing pad. This information was stored in a microcomputer and then replotted using an appropriate software package (Sigma Scan, Jandel Scientific, Sausalito, CA). In general, three to six records of a particular experiment were replotted on a much contracted horizontal axis, with points of averaged data occurring every 30 s. Standard deviations were calculated for these averages at 5 min intervals.

**RESULTS**

**Steady-State Measurements of Ion Distribution.** Figure 1 shows the apparent steady state distribution of H⁺, K⁺, and Ca²⁺ measured with ion selective electrodes positioned at tip and base locations near the apical cell of a fern gametophyte about 1 h after it was placed in unbuffered APW medium with 10⁻⁶ M concentrations of H⁺, Ca²⁺, and K⁺. From such measurements it was evident that the region near the tip of the apical cell was more acid than the base; however, as shown in Figure 2, both areas were more acid than the bulk medium at pH 6. Entry of K⁺ and Ca²⁺ into the tip region was inferred from the existence of K⁺ and Ca²⁺ depletion zones near the tip, with K⁺ depletion being the larger of these signals.

To determine how far these localized ion distributions extended into the bulk medium, we mapped the activity of these three cations at increasing distances from the surface of the apical cell at steady state. Figure 2 shows the distance-related decline in the ion accumulation (positive mV values) and depletion zones (negative mV values) as the electrode tips were positioned at 2, 5, 10, 15, and 20 µm from the tip and basal surfaces of the apical cell. The gradient in pH extended furthest from both the tip and base of the cell; this could either be due to a H⁺ flux that was larger than those of the other ions, or simply to the higher mobility of H⁺ in solution. In contrast, K⁺ differences could be

![Fig. 1. Steady state tracings of localized distributions of H⁺, K⁺, and Ca²⁺ within 2 µm of the tip and base regions of a dark-maintained apical cell. Upward deflections from the baseline (horizontal, broken line in each panel) indicate increases in the free concentration of ions. Arrows indicate the point at which the electrode was moved to either tip or base measuring positions. Medium concentrations of H⁺, K⁺, and Ca²⁺ were 10⁻⁶ M.](image)
detected no further than 15 \mu m from the cell and the Ca^{2+} activity profile was indistinguishable from the bulk medium at only 5 \mu m from the cell. The distribution of the medium acidity near the apical cell was mapped in greater detail by taking H^+ measurements at several positions around the cell, from which was constructed a diagram of the pH profile created by the cell (Fig. 3).

**Ion Measurements during the Restoration of Steady State.** Measurements of K^+, H^+, and Ca^{2+} ion activities within 5 s following a reinfusion of APW medium made up to equimolar concentrations of H^+, K^+, and Ca^{2+} at 10^{-6} M revealed a rapid increase in proton activity at the tip, apparently in response to the disruption of the region of localized acidity achieved at steady state (Fig. 4). Levels of K^+ and Ca^{2+} near the tip region decreased less markedly following the medium shift, suggesting a small influx of these ions was necessary to restore the steady state. The average pH change was about 1.65 mV/min (0.027 pH units/min, against a background of pH 6, with a 60 mV/decade response for this electrode). The rate of acidification measured near the base was much lower: 0.37 mV/min (0.006 pH/min). Measurement of K^+ activity near the gametophyte, following the infusion of fresh medium, revealed the development of a K^+ depletion zone at the tip (0.87 mV/min, 0.016 pK/min, with a 55 mV/decade response for this electrode) and a smaller depletion zone near the base (0.3 mV/min, 0.005 pK/min). Calcium activity was also slightly lowered near the tip region (0.25 mV/min, 0.006 pCa/min, assuming 33 mV/decade electrode response) but changed very little near the base when monitored immediately following medium replacement.

**Ion Redistribution following Blue Light Irradiation.** To examine how the rate of ion movement was affected by blue light, we carried out experiments, similar to the ones described above, in which we observed the restoration of steady state following medium replacement. In this case, however, the gametophytes were exposed to 2 min of blue light during the infusion of new medium. Averaged traces from a number of experiments involving blue light-treated gametophytes are shown in Figure 5. Comparison of the restoration of localized ion distributions in light-treated gametophytes with data from similar experiments performed in darkness (Fig. 4), revealed that the only significant changes in ion distribution occurred near the tip and involved H^+ and K^+. Irradiation of gametophytes with blue light lowered both the rate and magnitude of acidification near the tip. The brief exposure to light did not appear to affect the timing of these responses, as acidification of the tip region in both dark and light treated samples was observed as soon as the electrode was placed at the tip position. The rate and extent of depletion of K^+ from the tip was also decelerated in blue light, although, in the light treated organisms, there was a noticeable lag of about 2 min in the onset of the response.

**Extracellular Currents.** Collection of current density readings from measuring coordinates around the apical cell of many gametophytes indicated that, in darkness, this cell exhibited a tip-exit current (Fig. 6A). On the basis of the measurements made with ion selective electrodes above, we are inclined to believe this current is largely dependent on proton efflux. Weaker, inwardly directed cation currents were evident along most of the length of the lateral walls of the apical cell (Fig. 6A), suggesting that the cellular dipole was skewed towards the tip end of the cell. Figure 6B shows the position and magnitude of vectors calculated for the measuring coordinate data shown in Table I.

Irradiation of the apical cell with 2 min of blue light caused the mean steady current leaving the tip to increase slightly within 4 min (Table I). In the ensuing 10 min steady current at all coordinates closest to the cell declined to less than 10 nA/cm^2 (Table I). Continued slow decline in the tip current occurred up until 30 min following light treatment (Table I), at which time no further changes were seen over the next 30 min (data not shown).

**Ionic Basis of the Extracellular Currents.** We followed the effects of ion substitutions in the bathing media by monitoring net current and by recording the rate at which the localized zones of H^+, K^+, and Ca^{2+} ions were restored following the disruption...
of steady state with low \( H^+ \), low \( K^+ \), and low \( Ca^{2+} \), and low Cl\(^-\) containing media. By this approach it was possible to discern if a decrease in net current, during the reduction in medium concentration of a single ion, was directly attributable to the diminished external supply of the omitted ion, or involved an indirect, adverse effect on the rate of transport of a different ion.

Figure 7 shows the effect of APW media made with differing \( H^+ \), \( K^+ \), and \( Ca^{2+} \) concentrations on the steady state current measured near the tip of the gametophyte (position a). Tip measurements were selected because the high density of current lines at this position permits a reasonable approximation of total current to be made from exit current. Lowering of proton concentration from pH 5 to pH 8 had the greatest effect on the current with less than 20 nA/cm² detectable at pH 8. Decreasing \( K^+ \) concentration from \( 10^{-4} \) to \( 10^{-5} \) M caused a slight increase in the measured current from 76 to 86 nA/cm²; a further decrease to \( 10^{-7} \) M decreased the current to 66 nA/cm². Following each decrease in \( Ca^{2+} \) from \( 10^{-4} \) to \( 10^{-5} \) M, tip current increased by a small increment (about 5 nA/cm²). From these data it is clear that \( H^+ \) and, to a lesser extent, \( K^+ \) had the greatest influence on currents measured in the surrounding medium. The increase in current between pH 6 and pH 5 as well as the decrease between pH 6 and pH 8 may be explained by the strong dependence of the membrane potential on pH in green plant cells (25). Since the pH of the cytoplasm is expected to be about pH 7, external pH of 6 and 5 would successively hyperpolarize the membrane, whereas pH 8 would depolarize it.

The effect of \( K^+ \) is harder to interpret. Lowering \( K^+ \) from \( 10^{-4} \) to \( 10^{-5} \) M would tend to hyperpolarize the membrane of a typical plant cell (interior \( K^+ \) concentration of about 100 mm), which might cause the observed increase in current. However, the drop in current at \( 10^{-7} \) \( K^+ \) cannot be explained on this basis and may be an indication that the low availability of \( K^+ \) in the external medium compromises the ability of the ion transporter to extrude \( H^+ \). Since the effects of \( Ca^{2+} \) were small, it is possible that \( Ca^{2+} \) does not have a direct function in current generation in this organism. However, a conceivably serious limitation in the \( Ca^{2+} \) measurements is the lack of accessibility to the space between the cell wall and the outer surface of the plasma membrane, which contains the population of ions that are most relevant in determining the electrochemical gradients across the membrane. Although these ions are separated from the tips of ion selective electrodes by only the thickness of the wall (about 0.2 \( \mu m \)), the high density of fixed anion charges in this region
(6, 15) may conspire to create steep asymmetries in the distribution of exchangeable cations, such as Ca$^{2+}$.

The contributions of H$^+$ and K$^+$ fluxes to the measured currents in the gametophyte were examined further in experiments summarized in Table II. These data include rates of ion accumulation or depletion from zones near the tip and base of the cell, as well as steady tip current, when the cells were exposed to medium that had lowered concentration of H$^+$, K$^+$, Ca$^{2+}$, or Cl$^-$. Because of the logarithmic relationship between the voltage response of the electrode and the actual ion activity, it was necessary to mathematically transform each electrode response, measured as the change in mV/s, against a particular background of ion activities, into directly comparable changes in the free concentration of ions (the equation and assumptions for these calculations are shown in the footnotes to Table II). When cells were presented with a medium of high pH, the apparent rates of proton extrusion and potassium ion uptake through the tip and base regions were lowered and the current declined to about 30% of its value in APW 6. The converse experiment, with low K$^+$ in the medium, produced a similar result; that is, the rate of reacidification of the tip and base zones was reduced with low medium K$^+$. Exposure of the gametophyte to low Ca$^{2+}$ had little effect on tip current or on the rates of H$^+$ accumulation and K$^+$ depletion near the tip and base. We also found that removal of Cl$^-$ from the medium, by exchanging Cl$^-$ for SO$_4^{2-}$ in APW, had little effect on ion distributions or tip current.

**DISCUSSION**

**Ionic Currents during Tip Growth.** The endogenous electrical field around dark-maintained apical cells of the fern gametophyte was observed with a vibrating probe as net current leaving the tip and detected with ion selective electrodes as increased acidity and decreased K$^+$ just outside the tip. If protons released from the tip occurred as part of the mechanism which generated current, then these findings are simplest to interpret in terms of a concentration of active proton transporters, whose activity may be coupled to K$^+$ influx, in the tip region of the cell. Proton translocating ATPases have been well characterized in a number of other types of plant cells (25, 26) and appear to be the predominant active transport protein in the plasma membrane of all green and nongreen plant cells studied to date. It is not possible from the present studies, however, to be absolutely certain that the apparent, coordinated migrations of H$^+$ and K$^+$ are not the result of two, unrelated events, whose timing happens to be extraordinarily well correlated. For example, we cannot eliminate the possibility that the increase in tip acidity could be the result of vesicular release of acid during exocytosis.

We have adopted the view that acid release was accomplished by membrane pumping of protons for the following reasons: (a) The predicted outcome of such pumps operating in the gametophyte tip is that the tip of the apical cell would become hyperpolarized relative to the base, an expectation confirmed by earlier studies in which we simultaneously impaled the apical cell in the tip and base regions with two microelectrodes (22). (b) A portion of the charge carried by outwardly moving protons in many plant cells appears to be balanced by the influx of K$^+$, either by direct exchange of H$^+$ for K$^+$ in the pump molecule, or by the coupled diffusion of K$^+$ through a separate channel (21). During periods of enhanced proton extrusion in the gametophyte, we always noted higher rates of K$^+$ influx; and severe lowering of K$^+$ concentration in the medium lowered the rate of tip acidification and the outward tip current. The apparent interdependence of the H$^+$ and K$^+$ fluxes is a finding that is difficult to explain on the basis of a phenomenon such as the fusion of acidic-containing vesicles with the plasma membrane.

To the extent that more protons tend to leave and fewer K$^+$ tend to enter the tip of the cell, a net tip-exiting current should be generated. To determine how the extracellular currents measured with the vibrating probe compared with currents derived from our measurements of ion fluxes with ion selective electrodes, we performed two types of calculations. In the first, a mathematical estimate of net current was performed using the measurements of external, steady state concentrations of H$^+$ and K$^+$, and Fick’s law for diffusion in one dimension: $J = -D\Delta C / \Delta x$. For the purposes of these calculations, the data in Figure 2, showing H$^+$ accumulation and K$^+$ depletion at increasing distances from the tip, were assumed to be roughly linear. The voltage differences obtained from H$^+$ and K$^+$ ion selective electrodes at positions 2 and 20 $\mu$m from the cell surface were converted into molar concentrations and inserted into the equation as $\Delta C$. The distance component, $\Delta x$, was similarly provided by subtracting the more distal measuring site (20 $\mu$m) from the more proximal site (2 $\mu$m). The diffusion coefficients used for H$^+$ and K$^+$ in H$_2$O at 25°C were $3.4 \times 10^{-5}$ cm$^2$/s and $1.9 \times 10^{-5}$.
ELECTRIC AND IONIC GRADIENTS IN GAMETOPHYTES

Table I. Summary of Extracellular Currents around Gametophytes in Darkness and in Blue Light

<table>
<thead>
<tr>
<th>Coordinates</th>
<th>Probe Position</th>
<th>No. Observations</th>
<th>Average Current (nA/cm² ± SD)</th>
<th>Direction</th>
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<tr>
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<td>Normal</td>
<td>10</td>
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<td>16.1 ± 2.5</td>
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<td>Normal</td>
<td>7</td>
<td>3.6 ± 1.2</td>
<td>In</td>
</tr>
<tr>
<td>b₂</td>
<td>Normal</td>
<td>3</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>c₂</td>
<td>Normal</td>
<td>6</td>
<td>2.3 ± 0.7</td>
<td>In</td>
</tr>
<tr>
<td>d₂</td>
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<td>6</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Subapical</td>
<td>Normal</td>
<td>4</td>
<td>2.5 ± 1.6</td>
<td>In</td>
</tr>
<tr>
<td>cell</td>
<td>Normal</td>
<td>4</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parallel</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 min after blue light</td>
<td>6</td>
<td>83.4 ± 16.3</td>
<td>Out</td>
</tr>
<tr>
<td>a₁</td>
<td>Normal</td>
<td>6</td>
<td>17.1 ± 6.1</td>
<td>In</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>6</td>
<td>22.8 ± 5.0</td>
<td>In</td>
</tr>
<tr>
<td>b₁</td>
<td>Normal</td>
<td>6</td>
<td>65.3 ± 11.1</td>
<td>Out</td>
</tr>
<tr>
<td>c₁</td>
<td>Normal</td>
<td>6</td>
<td>9.3 ± 2.5</td>
<td>In</td>
</tr>
<tr>
<td>d₁</td>
<td>Normal</td>
<td>6</td>
<td>13.4 ± 3.4</td>
<td>In</td>
</tr>
<tr>
<td></td>
<td>Parallel</td>
<td>8</td>
<td>12.0 ± 3.5</td>
<td>Out</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>6</td>
<td>2.2 ± 0.7</td>
<td>In</td>
</tr>
<tr>
<td>a₁</td>
<td>Normal</td>
<td>6</td>
<td>7.8 ± 2.6</td>
<td>In</td>
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<tr>
<td></td>
<td>Normal</td>
<td>6</td>
<td>9.3 ± 2.6</td>
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<td>b₁</td>
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<td>4</td>
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</tr>
<tr>
<td>c₁</td>
<td>Normal</td>
<td>3</td>
<td>8.6 ± 1.1</td>
<td>In</td>
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</table>

cm²/s, respectively. The resulting calculated fluxes in mol/cm²-s, were converted into current by multiplying times Faraday's constant. For H⁺ gradients the current value was about 700 nA/cm² and for K⁺ gradients the current was about 400 nA/cm²; the difference between these values, 300 nA/cm², represented an average, net outward current. Since the ion selective electrodes could be placed much closer to the gametophyte than the vibrating probe, comparison of calculated and measured tip currents required that the data obtained with the more remotely placed vibrating probe be mathematically extrapolated to positions nearer the gametophyte surface. Using an equation for attenuation of current into the surrounding medium (20), we calculated that the 75 nA/cm² current measured at 20 μm from the tip would correspond to 166 nA/cm² at 10 μm and 347 nA/cm² at 2 μm from the cell surface. The favorable correspondence in the measured current values and those derived from measurements of external ion gradients implies that the currents in this system could be generated by ion diffusion in the external medium. This outcome is contrary to theoretical treatments of current detection by the vibrating probe, which predict that extracellular currents depend solely on transport events occurring in the cell membrane (10). To obtain some measure of the rates of ion passage across the region of membrane in the tip of the apical cell, we transformed the calculated data for ion fluxes (in ions/s) during attainment of steady state (Table II; H⁺ and K⁺ fluxes at the tip in APW medium) into current, assuming that 1 amper is equivalent to the movement of 10.4 μmol of charge-s⁻¹. For a volume of medium near the apical tip of 1 nl (this would be a sphere 5 μm in diameter), this equation predicts a current value at the membrane surface that is nearly 3 orders of magnitude lower than that actually measured with the probe. This finding suggests that most of the current is carried by another ion or that the current and fluxes of H⁺ and K⁺ are much higher in the region between the outside of the cell wall and the plasma membrane. The latter possibility, it seems to us, is not at all unreasonable in light of two additional observations which may be made about the gametophyte apical cell. First, earlier, direct measurements with intracellular microelectrodes (22) indicated that an apical cell, 100 μm in length, exhibited a tip to base polarization of 5 mV. Assuming a resistivity in the cytoplasm of
about 100 \Omega \text{ cm}^{-1}, this cellular field could generate a value of 5 mA/cm² for the cytoplasmic portion of what is, presumably, a single current loop. Second, as discussed below, the apical cell is capable of growth in the air and thus possesses some of the attributes of epidermal cells, including the presence of hydrophobic substances such as wax in the cell wall (29).

**Relationship of Electrical Changes to the Morphology of the Gametophyte.** The earliest detectable response to a blue light stimulus in the gametophyte cell was a hyperpolarization of the membrane in the tip of the cell that began within 1 to 5 s after the light was turned on and continued for the next 5 min (22). In the experiments reported here, the observation that steady current from the tip increased slightly during the 4 min after irradiation is consistent with the earlier findings, although it must be conceded that the magnitude of this change is near the threshold for noise in the system. Steady current from the tip began to decline about 5 min after blue light, and this event was well correlated with a steady decline in tip acidification during this period. The continued drop in current density eliminated the longitudinally aligned field around the tip growing cell within 10 to 15 min after light irradiation. As there was no concomitant decline in the rate of acidification or K⁺ depletion along the lateral walls of the apical cell, the ultimate effect of the light treatment was to create evenly distributed, shallow gradients of H⁺ and K⁺ over the cell surface.

A considerable body of evidence indicates that the structure of primary plant cell walls is regulated in large part by the attainment of low pH in the wall region (23, 27). In the dark-maintained gametophyte, the higher rate of proton extrusion in the tip should favor wall extensibility in this region and the influx

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**Table II. Electric and Ionic Parameters of the Dark-Maintained Gametophyte Apical Cell in Various Bathing Media**

<table>
<thead>
<tr>
<th>Medium⁵</th>
<th>Recordings from Ion Selective Electrodes</th>
<th>Recordings from Vibrating Probe Outward Tip Current</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tip</td>
<td>Base</td>
</tr>
<tr>
<td></td>
<td>H⁺</td>
<td>K⁺</td>
</tr>
<tr>
<td>APW</td>
<td>43 ± 5</td>
<td>-26 ± 8</td>
</tr>
<tr>
<td>Low H⁺</td>
<td>102 ± 14</td>
<td>-2 ± 2</td>
</tr>
<tr>
<td>Low K⁺</td>
<td>28 ± 6</td>
<td>-57 ± 6</td>
</tr>
<tr>
<td>Low Ca²⁺</td>
<td>41 ± 7</td>
<td>-23 ± 6</td>
</tr>
</tbody>
</table>

⁵ APW contains equimolar concentrations of H⁺, K⁺, and Ca²⁺ at 10⁻³ M; low H⁺ is APW medium with 10⁻³ M K⁺ and Ca²⁺, and 10⁻³ M H⁺; low K⁺ is APW medium with 10⁻³ M H⁺ and Ca²⁺, and 10⁻³ M K⁺; low Ca²⁺ is APW medium with 10⁻³ M H⁺ and K⁺, and 10⁻³ M Ca²⁺; and low Cl⁻ is the same as APW (with equimolar cations) except that it is made with sulfate rather than chloride salts. The upper values are the total voltage responses (mV ± sd for four determinations) over a 20 min recovery period following disruption of steady state (see “Results”). The lower values in parentheses are the calculated number of ions moving per second into (+ values), or out of (− values), a defined volume (+) of 1 picoliter surrounding the electrode tip, according to the following formula:

\[ x = (s \cdot v(10^{11}\text{ mol}) - 1)N_A \]

(x is rounded to 2 significant figures), where: \( V \) is the cellular response measured with ISE; \( E_s \) is the calibration factor for the ISE; s is the molar concentration of the ion being monitored in solution; \( N_A \) is Avogadro’s number. Negative values for voltage changes or calculated ion fluxes indicate a depletion of ions from the medium near the cell.
of K+ ions would augment the supply of osmotically active particles in the enlarged cell. Consistent with these predictions for the maintenance of tip growth, observations of a rapid diminution of proton efflux and outgoing current from the tip, following irradiation with blue light, suggests that a reapportionment of proton transport activity is an early, and perhaps crucial, step in the sequence of events which govern the change in shape which this cell undergoes. The resulting, more uniform distribution of proton pumping activity, evidenced by acidification of the lateral portions of the cell wall, would distinctly favor the ensuing period of isodiametric cell expansion.

Comparison with Other Tip-Growing Systems. The electric fields around the dark grown fern gametophyte, which appeared to arise as a consequence of net proton efflux from the tip of the apical cell, were oriented in the reverse direction as those described for a number of other tip-growing cells (reviewed in Refs. 4 and 14). It has been argued on the basis of the taxonomic and anatomical diversity of the tissues measured, that tip entrance currents, often composed of Ca2+ or H+, can be considered general characteristics of single-celled structures which exhibit tip growth. In our view, however, the comparison of systems solely on the basis of the direction of their electrical polarities may attach a disproportionate level of significance to the electrical manifestations of ion traffic in tip growing cells. The specific types of ion transport and the resulting asymmetries in ion distribution may be more critical factors in such polarized growth. Along these lines it is interesting to note that Ca2+ influx occurs at the tip of the gametophyte apical cell, despite the presence of outwardly directed net current. In addition, this apparent diversity of previously described experimental systems is deceptive because the cells in these organisms all have the specific task of absorbing nutrients from the surrounding medium (reviewed in Ref. 4). In certain examples, in fact, there appears to be a chemo- tropic character in the tip growth response in the presence of particular media-borne components. For instance, the rhizoids of fucoid zygotes will preferentially grow towards a concentrated source of the calcium ionophore A23187 (24). In addition, the hyphae of the water mold Achlya appear to use an inward proton flux to cotransport methionine, an amino acid essential for its growth (16).

On the other hand, the apical cell of the photosynthetic fern filament exhibits positive phototropic growth (7, 9), with the rate of tip extension being relatively insensitive to theionic conditions in the medium (17; TJ Cooke, unpublished observations). The fate of the gametophyte apical cell is to become an aerial structure which assumes a rather complex two-dimensional morphology, whereas root hairs, fungal hyphae, algal rhizoids, and pollen tubes do not undergo major changes in form, nor are they ever capable of departing from an aqueous environment. For these reasons it is probable that an aerial, photosynthetic, tip-growing structure like the fern gametophyte must conform to a more complex set of developmental cues in a dissecting environment, where solvated ions may be in limited supply. Our observations suggest that the existence of an electric field along the dark-grown apical cell of the gametophyte is secondary in importance to the underlying ion transport events, which are responsible for gradients of pH, turgor, and, possibly, cytoplasmic calcium that contribute to the phenomenon of tip growth.

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