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Inversion of Extracellular Current and Axial Voltage Profile in *Chara* and *Nitella*

Joachim Fisahn and William J. Lucas
Department of Botany, University of California at Davis, Davis, California 95616

Summary. Reducing the pH of the bathing solution from 8.2 to pH 6 can induce an inversion of the extracellular current pattern that develops at the surface of *Chara corallina* internodal cells. A similar result can be obtained on some cells by changing the medium to a pH value of 10. In noninverting *Chara* cells the currents were strongly reduced when the pH value of the medium was changed between 3 and 11. Simultaneous measurements of the *Chara* transmembrane potential in the acid and alkaline regions revealed that a light-induced electrical potential gradient of approximately 24 mV was present in the axial (or longitudinal) direction. Correlated to the external current pattern inversion was an inversion of this internal longitudinal voltage gradient. Reillumination of *Nitella* cells, after a period of darkness, often resulted in a complete inversion of the extracellular current pattern. These results are discussed in terms of spatial and temporal control of membrane transport processes, and in particular the control of current loops that pass through these cells.

Key Words vibrating probe · *Chara* · *Nitella* · currrent inversion

Introduction

The freshwater algae *Chara corallina* and *Nitella flexilis* develop alternating alkaline and acid regions along their internodal cells, when exposed to light (Lucas & Smith, 1973; Lucas, 1975; Lucas & Nuccitelli, 1980; Lucas, 1982). Fisahn, McConnaughey and Lucas (1989) described areas in the banding pattern that were able to change their assignment from acid to alkaline behavior during activation following reillumination. Lucas (1979) has also shown that it is possible, under various conditions, to induce extracellular acidification in an otherwise alkaline region. In this paper a complete inversion of the banding structure is demonstrated.

Walker and Smith (1977) proposed that a current loop exists between the acid and alkaline regions. In their model they assumed that a complicated current distribution was present between the vacuole and the cytoplasm. Lucas (1983) suggested a model that postulated a flow of OH^- ions between the acid and alkaline regions within the cytoplasm. Internal components (in the vacuole or the cytoplasm) of a circulating current should be detectable by a voltage drop along the axial, or longitudinal direction of the cell. This prediction of the circulating current model was tested by conducting simultaneous measurements of potentials, in the vacuole, in an acid and an alkaline band. These results confirmed the existence of components of this current loop within the vacuole. Additionally, the longitudinal voltage gradient was used to further elucidate the mechanisms responsible for the inversion of the extracellular current pattern.

Materials and Methods

Culture Material

Culture material of *Chara corallina* Klein ex Willd., em. R.D.W. (=*C. australis* R.Br.) was grown in the laboratory in 120-liter containers in solutions containing (in mM): 3.0 Na^+, 0.3 K^+, 0.2 Ca^{2+}, 1.5 Cl^- and approximately 2.0 HCO_3^- (pH 9.0). Light from fluorescent lamps (cool-white, Lifeline; Sylvania, Seneca Falls, NY) were employed; the irradiance at the solution surface was 20 Wm^{-2} (measured using a silicon solar cell, Type No SO51OE 7PL; International Rectifier, Los Angeles, CA).

Nitella flexilis was collected from rice fields near Davis, CA and maintained under the same conditions as *C. corallina*.

Extracellular-Current Measurements

Extracellular currents near the cell wall of internodal cells of *C. corallina* and *N. flexilis* were measured by using an improved vibrating probe system (Nuccitelli, 1986). The protocol for the vibrating probe experiments has been described by Fisahn et al. (1989). The tip of the voltage sensing electrode was generally 10 μm in diam., and this electrode was vibrated at a frequency of 294 Hz. The reference electrode was simply a large stationary Pt probe (100 μm in diam.) located 3 mm away from the point of current detection.

Chara or *Nitella* internodal cells were mounted in Plexiglas chambers and placed on the stage of a Zeiss IM 405 inverted

Table 1. Current densities ($\mu A/cm^2$) in acid and alkaline regions of 12 *Chara corallina* cells that were inverted following a solution change from CPW/B (pH 8.2) to CPW/B (10 mM MES, pH 6.0)[a]

	Control currents in CPW/B (pH 8.2)		Inverted currents in CPW/B (pH 6)		Transition time (min)
	Outward[a]	Inward	Outward	Inward	
Mean	−15.8	59.05	−27.09	10.30	18.1
SD	11.1	33.19	22.30	8.20	13.53

[a] Values represent the mean of maximum amplitudes ($\mu A/cm^2$) measured in the regions of outward (H^+ efflux or acid region) and inward (H^+ influx or alkaline region) current.

microscope (*see* Lucas, 1982). For a quick calibration and polarity check of the vibrating probe system, two Ag/AgCl electrodes could be dipped into the bathing solution at one end of the Plexiglas chamber. These electrodes were connected to a 1.5-V battery via a 1 MΩ resistor. Before starting an experiment, and after getting the pattern inversions described later, the test for correct functioning of the vibrating probe system was performed to eliminate the possibility of instrument-associated artifacts.

A Schott KL 1500 fiber optics light source was used (bulb made by Philips EFR Al/232) to illuminate cells during vibrating probe and microelectrode measurements. For relaxation measurements, light intensity was switched between 20 Wm^{-2} and darkness.

All experiments were conducted in temperature-controlled rooms at 25 ± 1°C.

Simultaneous Potential Measurement In Acid and Alkaline Regions

Two glass microelectrodes filled with 3 M KCl were inserted, simultaneously, into the vacuole of an internodal cell, with one being located in an acid band, and the other one in the neighboring alkaline band. A WPI amplifier (Model 750 dual microprobe) was used for impedance adjustment and amplification of the microelectrode signal. Membrane potential recordings from the two microelectrodes were stored on both an IBM AT computer and an HP (model 7132A) chart recorder. Analog-to-digital conversion was performed by the IBM Data acquisition and control adapter, controlled by the notebook 2.7 program from Laboratory Technologies (Wilmington, MA).

Experimental Solutions

Experimental medium contained 1 mM NaCl, 0.2 mM KCl, 0.2 mM $CaCl_2$ and 1 mM $NaHCO_3$ (CPW/B). When the pH was changed to 5 or 6, 10 mM MES was added to the CPW/B and the pH was adjusted with NaOH. In the range of pH 10 and 11, CAPS buffer (10 mM) was used. When solutions having pH 3 or 4 were employed, the pH value was obtained by titrating CPW/B using HCl, and no artificial buffer was included. In this situation frequent changes in bathing medium ensured that the pH value remained constant. Control experiments with unbuffered solutions resulted in the same effects.

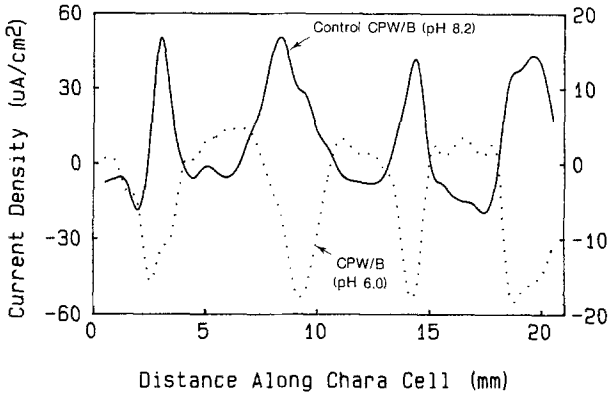

Fig. 1. Acidic pH-induced inversion of the extracellular current pattern that develops at the surface of internodal cells of *Chara corallina*. The current profile measured in CPW/B (pH 8.2) represents the control pattern (solid line, with scale indicated on left current axis). Changing the bathing medium to CPW/B (pH 6, buffered with 10 mM MES) elicited an inversion of the current pattern (dotted line, with scale indicated on the right current axis)

Results

Extracellular Current Inversion In *Chara* By Acidification of the External Medium

Under photosynthetic conditions, alternating acid and alkaline bands develop along internodal cells of *Chara* (Lucas & Smith, 1973). We have also established that these acid and alkaline regions correlate with areas of extracellular negative and positive current, respectively (Lucas & Nuccitelli, 1980; Lucas, 1982). In the present study we show that decreasing the pH of the CPW/B to values between 5 and 6 can cause an inversion of these extracellular current patterns (Fig. 1). A period of approximately 18 min was required to establish the inverted current pattern (Table 1), and in this state, the amplitude of the current was reduced compared with that obtained under control (CPW/B, pH 8.2) conditions. However, a consistent feature of the inverted state was that currents detected in the acid regions were stronger than those measured in the inverted alkaline regions (Fig. 1, *see also* Table 1). These acidic pH-induced inverted current patterns could be returned to normal control profiles simply by changing the medium back to CPW/B (pH 8.2).

Membrane potential measurements, made using a single inserted microelectrode, revealed that changes from CPW/B (pH 8.2) to CPW/B (pH 6.0) resulted in only minor voltage differences (a few mV) between the initial and the inverted states (*data not shown*).

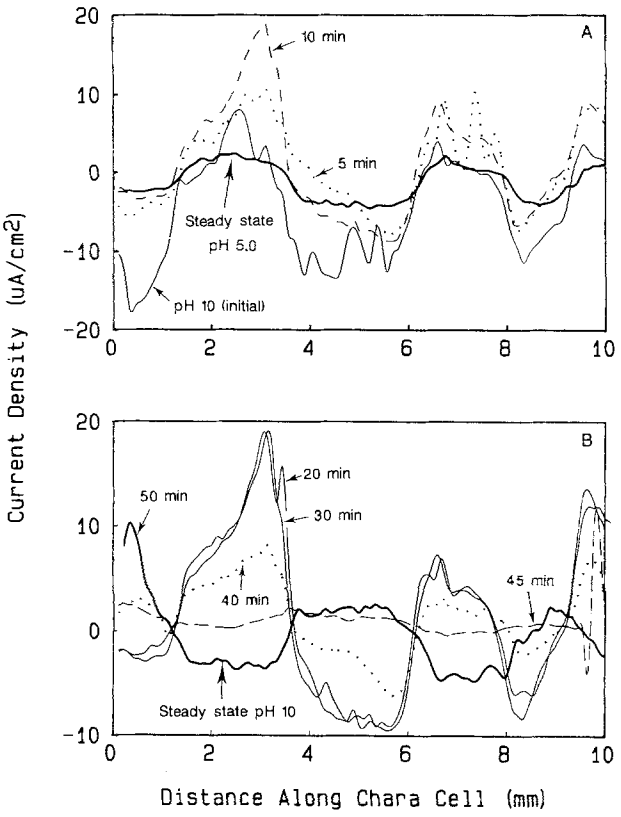

Fig. 2. Time course for the inversion of the extracellular current pattern in *Chara* elicited when the bathing medium was changed from CPW/B (10 mM MES pH 5.0) to CPW/B (10 mM CAPS pH 10.0). Steady-state currents in the acid and alkaline regions of this CPW/B (pH 5.0)-treated cell were strongly reduced, as this cell did not undergo an acid pH-induced inversion of its pattern

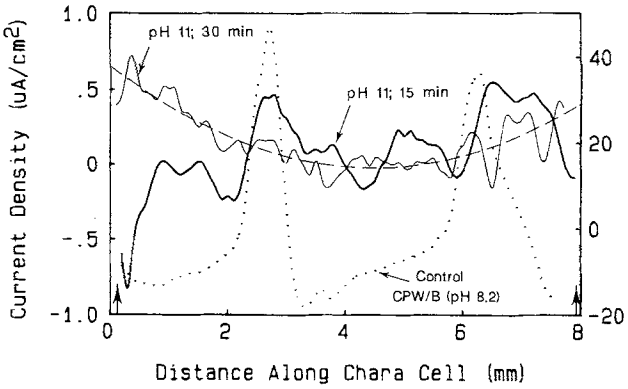

Fig. 3. Time course of the extracellular current pattern obtained on a *Chara* internodal cell that was transferred from CPW/B (pH 8.2) to CPW/B (pH 11.0). Note the dramatic reduction in the magnitude of the extracellular currents under CPW/B (pH 11.0) conditions (*see* left current axis). A regression analysis was performed on the current data collected after 30-min exposure to CPW/B (pH 11.0) (*see* long dashed line). (Vertical arrows on *x*-axis represent location of the nodes)

Time Course of Alkaline-Induced Current Inversion

Out of 32 cells investigated, 12 exhibited an inversion of the current pattern when transferred to CPW/B (pH 6). However, of the noninverted cells, five responded with an inversion of their current patterns when exposed to pH 10 (CPW/B + 10 mM CAPS). In these experiments, the currents in the acid and alkaline regions were strongly reduced when the cells were exposed to pH 5 (*see* Fig. 2*A*). Immediately after raising the pH of the bathing solution from pH 5 to 10.0, the outward current regions showed considerable increases, resulting in an overall net outward current. Pretreatment of these *Chara* cells at pH 5.0 would have caused a lowering of the cytoplasmic pH value, and thus, when the external pH was changed to 10.0, the thermodynamic gradient for H^+ movement would be consistent with these currents. The immediate consequence would be a time-dependent increase in cytoplasmic pH.

After 5 min at pH 10 the outward currents along the *Chara* cell surface decreased, whereas the inward currents increased (Fig. 2*A*). The extracellular current pattern then remained stable over the 10–30 min period after transfer to pH 10.0 medium (Fig. 2*A* and *B*). Then, after 40 min at pH 10.0, the pattern suddenly started to collapse, and over the next 10-min period the currents underwent an inversion. The extracellular current pattern measured at the 50-min time point remained stable for a further 70 min. Returning these inverted cells to CPW/B (pH 5.0) resulted in the reestablishment of their original current pattern. Membrane potential changes during these inversions were also minor (*see* later section).

Current Profiles of Noninverted Cells

Cells which could not be inverted by long-term treatments ($\geq$1 hr) at pH 6 or 10, were exposed to a range of pH values from pH 3 to 11. Cyclosis was observed to be almost unchanged at the extremes of pH values employed. Only during the initial 10 to 20 min after the change in pH 11 did we observe an effect on cyclosis; the rate was reduced to approximately 50% and then it recovered to control values. Figure 3 illustrates that a dramatic change occurred in the extracellular current pattern when noninverting cells were transferred from CPW/B (pH 8.2) to CPW/B (pH 11.0). After a 15-min exposure to CPW/B (pH 11.0), the entire current pattern had become drastically reduced. Long-term exposure to pH 11 resulted in an extracellular current pattern in

Table 2. Current densities ($\mu A/cm^2$) in acid and alkaline regions of five *Chara corallina* cells in which the extracellular current pattern did not invert when the cells were exposed, for 1 hr, to CPW/B (pH 5.0) or CPW/B (pH 10.0) conditions[a]

	CPW/B control (8.2)	pH Value of the experimental medium					
		11	10	6	5	4	3
Mean of outward current (acid)	−9.25	−0.83	−3.8	−1.2	−3	−0.5	−2.5
SD	2.2	0.2	3.6	0.5	1.3	0.1	1.5
Mean of inward current (alkaline)	67	2.5	11.8	3	1.9	2.5	5.5
SD	25.8	1.5	10.7	2.9	1	1	2.5

[a] Following this pretreatment, cells were treated with CPW/B solutions in which the pH value had been adjusted over the range pH 3 to 11 (*see* text for details).

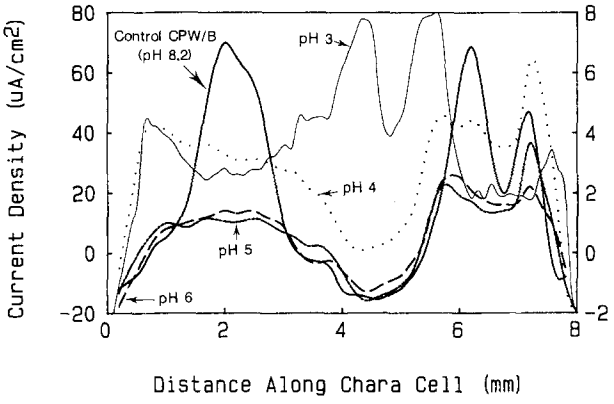

Fig. 4. Current profiles obtained when *Chara* cells were transferred from CPW/B (pH 8.2) to various pH values (indicated). Cells were given 10-min treatment at the specified pH value before current patterns were measured. Each low pH solution treatment was followed by 30 min in CPW/B (pH 8.2). The left current scale refers to the control pattern in CPW/B (pH 8.2). The right current scale refers to all low pH current profiles. Averaged data are provided in Table 2

which almost the entire cell surface had positive values with the extreme values being close to the nodes. (The long dashed line in Fig. 3 represents a regression line through the final current pattern, as the bumps in this small current distribution might have been caused by the measuring procedure of adjusting the focus during a run along the cell.) Returning these pH 11-treated *Chara* cells to CPW/B required 3–4 hr before control patterns were reestablished.

Figure 4 shows the response obtained when noninverting cells were subjected to a sequential decrease in the pH value from 8.2 to 3.0. In these experiments, *Chara* internodal cells were transferred from CPW/B (pH 8.2) to CPW/B in which the pH value had been successively lowered. Following each low-pH treatment the cell was returned to CPW/B (pH 8.2) for 30 min. At pH 6 and 5 the current patterns were almost identical, and the reduced values are consistent with the data presented in Fig. 2*A*. Note that the areas close to the nodes always reflect negative (or outward) current regions. Exposing the cell to pH 4 and 3 resulted in a successive increase in the inward (positive) currents and, under these conditions, most of the cell surface apparently had positive current, except the nodal regions. It should be noted that the original area of outward current at 4.5 mm (control pattern) becomes the strongest region of inward current at pH 3. Table 2 contains the statistics of these pH experiments performed on five noninverting *Chara* cells.

Simultaneous Measurement of Potential in an Acid and Alkaline Band

As the internal current loop represents an important aspect of the hypothesis developed to explain the pH banding phenomenon, it was essential for us to establish that voltage gradients exist along our *Chara* cells. Figure 5 illustrates the response of the membrane potential in an acid and a neighboring alkaline region when the *Chara* cell was switched between darkness and 20 W/m^2. Under steady-state conditions, in the light, a 20–25 mV voltage difference was detected between these regions, with the acid region being more negative. This internal electric gradient remained as long as the cell was illuminated. Turning off the light led to a strong hyperpolarization of up to 100 mV, followed by a slow depolarization. The longitudinal potential difference between the acid and neighboring alkaline regions disappeared completely in the dark. Immediately after switching the light on, a small hyperpolarization was observed, followed by a strong depolarization of up to 100 mV. After reaching a

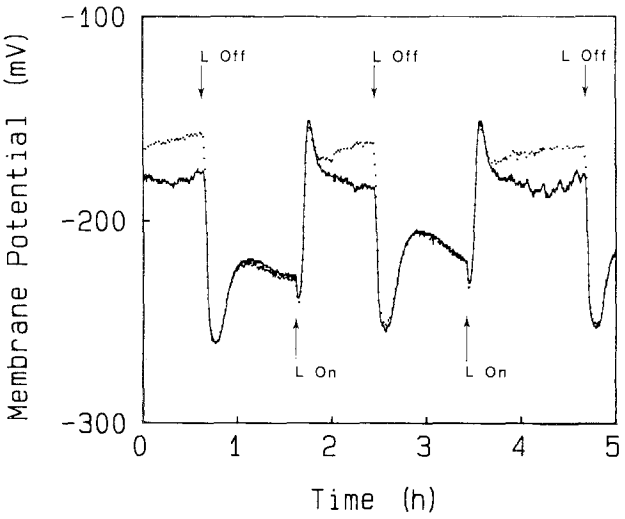

Fig. 5. Time course of the membrane potential (light/dark responses) in the acid (solid line) and alkaline (dotted) regions of a *Chara* internodal cell measured simultaneously with microelectrodes located in an acid and a neighboring alkaline region. Note the significant longitudinal gradient present in the light, which then disappears in the dark

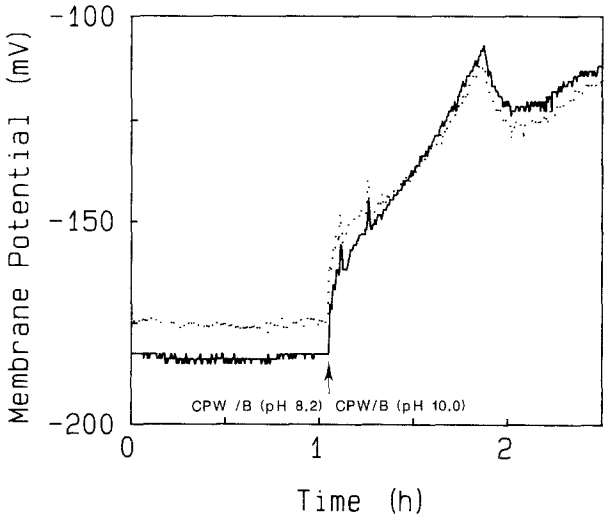

Fig. 6. Inversion of the internal longitudinal electric potential gradient of a *Chara* internodal cell whose extracellular current pattern was inverted in CPW/B (pH 10). Experimental details as in Fig. 5. Initially, the *Chara* cell was bathed in CPW/B (pH 8.2) and then the medium was changed to CPW/B (pH 10). Note that after approximately 20 min the internal electric gradient changed its sign

maximal depolarization a slow hyperpolarization occurred, and during this phase, the electrical potential gradient of approximately 25 mV was reestablished between the two areas, with the potential again being less negative in the alkaline band (*see* Fig. 5).

The mean value of the potential differences observed between the acid and alkaline region in 30 light/dark experiments performed on five *Chara* cells, was 24 (±9) mV (Table 3). It should be noted that the initial changes, especially the strong depolarization following light on, occurred simultaneously in both regions. Furthermore, as the activation and deactivation of the acid and alkaline regions differed in their initial phases, these events cannot be described by linear kinetics.

Table 3. Longitudinal voltage differences (V_l) between acid and alkaline regions and transmembrane voltage gradients (E_m) under different light/dark and bathing media pH conditions

CPW/B	V_l (mV)			Light/dark-induced changes in E_m (mV)		
	Mean	SD	n*	Mean	SD	n
pH 8.2	24	9	30	—	—	—
pH 10	6.3	3.2	5	6.1	2.3	5
pH 5	—	—	—	4.9	1.8	5

* n = number of replicate experiments.

Inversion of Longitudinal Electrical Potential Gradient

When the extracellular current pattern was inverted, by exposing *Chara* internodal cells to CPW/B (pH 6), we observed only minor changes in the transmembrane potential. However, cells that were inverted when exposed to CPW/B (pH 10) did show a significant change in their membrane potential (Fig. 6). Changing the pH of the bathing solution from pH 8.2 to 10 resulted in a gradual depolarization of the membrane potential and, after approximately 20 min, the internal electrical potential gradient had changed in sign. The absolute magnitude of the axial electric gradient was reduced under CPW/B (pH 10) conditions, and this result is consistent with the reduced, inverted, extracellular current pattern measured under these conditions. Returning the cell to CPW/B (pH 8.2) resulted in the reestablishment of the control axial internal electric gradient after approximately 20 min (*data not shown*).

Light-Induced Changes In Transmembrane Potential

Cells in which an inversion had been induced by exposure to CPW/B (pH 10) were next used to investigate the effect of light/dark treatment on the membrane potential. At pH 10 these cells hyperpo-

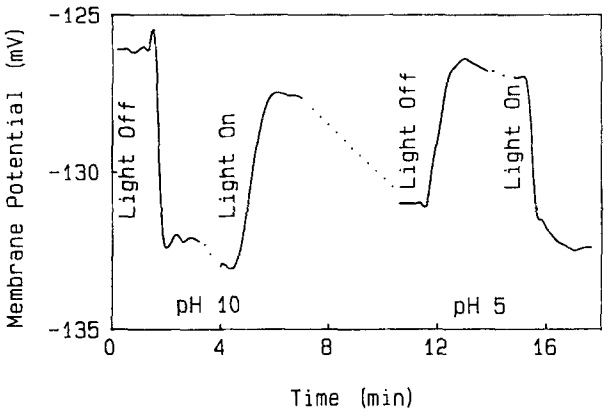

Fig. 7. Light effect on the membrane potential of a *Chara* internodal cell in which the extracellular current pattern was inverted at pH 10, relative to pH 5.0. The light off responses are of opposite sign in the two states, initial and inverted. The dotted line represents a 2-hr pretreatment period at CPW/B (pH 5) that was employed to ensure that the cell had inverted to the low pH state prior to conducting dark/light treatments. A small drift in the membrane potential was observed during this transition period. (In each light off treatment, the *Chara* cell was kept in the dark for a period of 15 min)

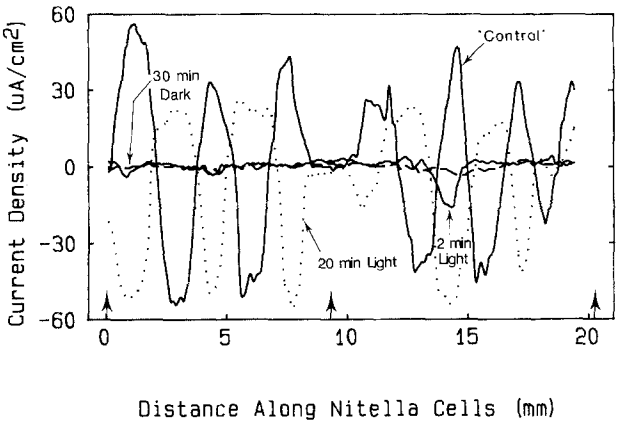

Fig. 8. Inversion of the extracellular current profile of a *Nitella* internodal cell during membrane transport reactivation by light. The "control" pattern represents the current profile developed in the previous dark/light transition experiment. Pattern deactivation is evident after 30 min in the dark. Two min after light on, a single acid region started to activate at the 14-mm position. Twenty min after reillumination the extracellular current pattern was activated, but with an inverted polarity. Two adjoining *Nitella* cells were used in this experiment; nodes are indicated by arrows

larized when the light was turned off (Fig. 7 and Table 3). After switching the light on again the membrane potential depolarized to a value close to that present before the application of the dark treatment. Cells were then kept in the light for approximately 1 hr. These cells were then pretreated for a period of 2 hr in CPW/B (pH 5.0) to secure the transition into their prior current pattern. During this transition phase from pH 10.0 to 5.0, the membrane potential remained almost constant. In the pH 5.0 state, light off now elicited a depolarization of the membrane potential (Fig. 7 and Table 3). These light/dark results indicated that the membrane potential responses were small in magnitude and opposite in sign at pH 5 and 10.

Inversion of Current Pattern in *Nitella*

The acid and alkaline banding phenomenon also occurs in species of the genus *Nitella* (Spear, Barr & Barr, 1969; Franceschi & Lucas, 1980; Fisahn et al., 1989). During recent vibrating probe experiments on *Nitella flexilis*, we found that different time constants in specific acid and alkaline regions dominated the establishment of the light-dependent current pattern. In the course of these studies we found that reillumination of *Nitella* cells, after a period of darkness, can cause an inversion of the currents along the cell surface. In this situation endogenous control processes must be responsible for the inversion.

A typical example of these results is presented in Fig. 8; the control pattern was stable as long as the cell remained illuminated (observed for several hours). After 30 min of darkness the bands were completely inactivated. Reillumination of the cell resulted in a time-dependent reactivation of the current pattern. The first detectable event, after 2 min in the light, was the activation of a single acid band at the 14-mm position. Note that in the control, this region functioned as an area of inward current (alkaline region).

Twenty min after reillumination, the current pattern was almost fully reactivated, but with an inverted pattern (dotted line). This pattern remained stable, in this inverted state, for many hours.

In the present study we used only *Nitella* cells that activated their acid regions prior to the alkaline bands (*see* Fisahn et al., 1989), and in this population of cells, six out of 10 responded with a complete inversion of the current pattern.

Discussion

In attempting to understand the inversion process documented for *Chara* (*see* Fig. 1), the transporter activities in the acid and alkaline regions will have to be reanalyzed. The banding structure observed in normal CPW/B (pH 8.2) is orchestrated by a complex cellular control system (*see* Fisahn et al., 1989). One observable effect of this control system

is the alternating pH distribution along the cell (Lucas & Smith, 1973; Lucas, 1982, 1983). Measurements of the pH along the cell surface indicate values of approximately pH 10 to 10.5 in the alkaline band and pH 6–8 in the acid bands, depending on experimental conditions and methods of measurement (Lucas, 1975; Lucas, Keifer & Sanders, 1983).

At present we consider that the activities of the transporters in these regions are determined by the internal control system and the concentration of the transported substrate(s) in the *cytoplasm* and the outer *surface* of the plasma membrane. According to Lucas (1983), a H^+-ATPase is responsible for the current and the pH in the acid band, whereas an OH^- (or H^+) transporter dominates the alkaline regions. As shown by Fisahn et al. (1989) and Bisson and Walker (1980), both types of transporters are present in the two areas, but activated to different levels. The mutual interaction between the internal transporter control system and the external pH of the bathing solution must result in a very well-defined state of transport activity. Changing the pH of the bathing solution over the range of pH 3 to 11 will thus result in a change in the banding structure, as indicated by different currents through the transporters (Smith & Walker, 1985).

Acidification of the bathing solution of pH 6 causes an extreme change in the conditions in the alkaline band, as the H^+ concentration in this area is affected by a factor of approximately 10^4. The acid regions will experience a rather small change in external proton concentration under the imposition of CPW/B (pH 6.0). We propose that in the alkaline band the H^+-ATPase is activated, by this change in the outside pH, to gain relations similar to those in the acid band. This activation of the H^+-ATPase in the alkaline region would affect the internal current loop between the two bands. Less protons would be transported to the acid area (or OH^- to the alkaline area), causing an increase of the cytoplasmic pH in the regions in which acid bands were initially present (control pattern). As a consequence, the proton channel (leak) in this area becomes activated, resulting in a slight alkalinization at the outer surface of the cell. The above-described hypothesis indicates one possible mechanism by which the acid and alkaline bands might change their polarity. What is not clear to us at present, is why all *Chara* cells *do not* undergo this inversion phenomenon. Resolution of this question will require that we obtain more details on the cellular control system.

Detection of the axial voltage gradients, within the vacuole of *C. corallina*, between acid and neighboring alkaline regions (Fig. 5) provides unequivocal support for the hypothesis that current loops are part of the banding phenomenon. Furthermore, the data presented in Fig. 6 offers strong support for our proposed model of the inversion process, in that the change in direction of the internal voltage gradient occurs within the same time frame as the inversion of extracellular current pattern.

The light/dark-induced changes in the membrane potential, obtained on cells under noninverting and inverting conditions, are also consistent with the pH-induced inversion of the extracellular current pattern (*see* Figs. 2 and 7).

Inversions induced by the reillumination of the *Nitella* cells (*see* Fig. 8) can probably be explained by the same general mechanism propounded to account for the externally triggered inversion phenomenon in *Chara*. Changing the light intensity that is used to illuminate characean cells results in a change of the internal cytoplasmic pH (Mimura & Kirino, 1984). The polarity of the current pattern in *Nitella* may be dependent on the momentary value of the internal cytoplasmic pH, in a manner similar to the outside pH sensitivity here reported for *Chara*.

The involvement of the $2H^+$-Cl^- cotransporter in the banding phenomenon can be ruled out on the basis of recent experiments in which it was found that the complete removal of Cl^- from the bathing solution did not influence the steady-state current pattern (J. Fisahn & W.J. Lucas, *unpublished results*).

Implications of the Inversion Phenomenon

Fisahn and Hansen (1986) identified a complex zero in the transfer function of the temperature-induced responses of the membrane potential, which was also found by Hansen (1978) in the light effect on the membrane potential of characean cells. Modulating the light intensity with a frequency of about 10 min resulted in almost no light response. In view of the observed pattern inversion, this zero might be identified with the same mechanism that caused the inversion of the *Nitella* current distribution.

To gain a more detailed understanding of the inversion process, and in particular, of the correlated transitions occurring in the plasma membrane, reaction kinetic modeling of the involved transporter(s) and its concentration dependence has to be performed. Initial computer simulations of a reaction kinetic model (Hill, 1985) comprising a pump and a channel (leak) are consistent with the observed inversion phenomenon.

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