Ion flux interaction with cytoplasmic streaming in branchlets of *Chara australis*

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Abstract

Both parts of the actin–myosin complex involved in cytoplasmic streaming could be regulated by mineral ions. The main goal of this study was to find a relationship between cyclosis and ion transport across the cell wall and plasma membrane. The transport of K\(^{+}\) and Ca\(^{2+}\) along pH bands in *Chara* branchlet internodal cells was characterized by using the MIFE system for non-invasive microelectrode measurement of ion fluxes. Branchlets formed acidic and alkaline bands with the pH ranging from 5 to 8. Different pH patterns were observed for different sides of the branchlets. Sides with cyclosis streaming acropetally generally showed greater variation in the profiles of pH and H\(^{+}\) fluxes.

Although a high correlation was not found between pH bands and Ca\(^{2+}\) or K\(^{+}\) fluxes, there was a positive correlation between Ca\(^{2+}\) and K\(^{+}\) fluxes themselves for both sides of the branchlets. Application of cytochalasin D, an inhibitor of cyclosis, had no immediate effect on pH and ion fluxes, however, the time of cyclosis cessation corresponded with a dramatic change in Ca\(^{2+}\) and K\(^{+}\) fluxes; pH profiles and H\(^{+}\) fluxes were affected within 2 h. The evidence suggests that, in *Chara* branchlets, pH band formation and Gd\(^{3+}\)-insensitive Ca\(^{2+}\) transport systems are linked to the cyclosis machinery: (i) the pH band amplitude for the acropetally streaming side was larger than that for the basipetally streaming side; (ii) cessation of cytoplasmic streaming after cytochalasin D application resulted in changed pH banding profiles and H\(^{+}\), Ca\(^{2+}\) and K\(^{+}\) fluxes; and (iii) the application of GdCl\(_3\) or incubation in GdCl\(_3\) solutions did not lead to the cessation of cytoplasmic streaming, although external Ca\(^{2+}\) fluxes changed.

Key words: Calcium, cyclosis, cytochalasin D, pH, potassium, proton.

Introduction

Most eukaryotic cells show cytoplasmic streaming. The motive force for streaming is generated by the sliding movement of myosin with bound vesicles, along the bundle of actin filaments, using energy derived from ATP hydrolysis (Alberts *et al*., 1994; Shimmen and Yokota, 1994). *Chara* cells show very fast endoplasmic streaming, probably because of the properties of their myosins, the fastest (60 μm s\(^{-1}\)) of all plant myosins observed so far (Morimatsu *et al*., 2000). Cytoplasmic streaming is obviously linked with the metabolic production of ATP for myosin movement and for actin polymerization. The link between cytoplasmic streaming and ion transport is less apparent. Reviewing the literature, Hope and Walker (1975) noticed that a temporary halt in cyclosis was caused by all stimuli that produce an action potential (mechanical shock, bending, and a sudden drop in temperature). They suggested that this could be linked with Ca\(^{2+}\) influx into the cytoplasm, however, at that time there was no direct evidence of a link between \([\text{Ca}^{2+}]_{\text{cyt}}\) and cyclosis. It has since been confirmed that \([\text{Ca}^{2+}]_{\text{cyt}}\) is increased during action potentials (Williamson and Ashley, 1982; Hayashi and Takagi, 2003).

Because six binding sites for calmodulin have been found in *Chara* myosins, it is plausible that Ca\(^{2+}\) can participate in regulating myosin action (Morimatsu *et al*., 2000). However, there is some controversy over Ca\(^{2+}\) involvement in myosin action. In an *in vitro* motility assay, *Chara* myosin did not show Ca\(^{2+}\) sensitivity (Higashi-Fujime *et al*., 1995), but *in vitro* motile activity of myosin isolated...
from the pollen tubes of lily was inhibited by Ca\(^{2+}\) (Yokota et al., 1999).

Changes in \([\text{Ca}^{2+}]_{\text{cyt}}\) are not the only events observed during action potentials that might cause cytoplasmic streaming to stop. Activation of Cl\(^{-}\) and K\(^{+}\) channels also contribute to the action potential (Oda, 1976; Hayama et al., 1979; Shimmen et al., 1994). Hence, temporary cyclosis cessation is linked with changes in cytoplasmic concentrations of at least three mineral ions: Ca\(^{2+}\), K\(^{+}\), and Cl\(^{-}\).

Involvement of K\(^{+}\) and Cl\(^{-}\) in the action potential could be of secondary origin as a consequence of plasma membrane depolarization or increased \([\text{Ca}^{2+}]_{\text{cyt}}\). However, there is some indication that K\(^{+}\) could be directly involved in the regulation of cytoplasmic streaming. Actin filaments need to be constantly polymerized from their plus end at a certain rate; any disruption in polymerization causes disruption in their functions, including cyclosis. Polymerization of pure actin \textit{in vitro} requires ATP, as well as both monovalent and divalent cations, usually K\(^{+}\) and Mg\(^{2+}\) (Alberts et al., 1994).

Thus, both parts of the actin–myosin complex involved in cytoplasmic streaming could be regulated by mineral ions, whose transport would therefore change during the cessation of cyclosis. The main goal of this study was to find a relationship between cyclosis and ion transport across the cell wall and plasma membrane. Light microscopy and the MIFE system for non-invasive microelectrode measurement of ion fluxes were used: (i) to characterize the transport of H\(^{+}\), K\(^{+}\), and Ca\(^{2+}\) along acidic and alkaline bands in branchlet cells and (ii) to compare the ion transport of the branchlets and the nodes before and after inhibition of cyclosis by cytochalasin D (CD) and inhibition of Ca\(^{2+}\) influx by GdCl\(_3\) treatment.

### Materials and methods

#### Plant material

*Chara australis* R. Br. cells were taken from an established indoor culture grown in a plastic tank. Individual branchlet cells with attached nodes or isolated branchlet nodes, as indicated in Fig. 1, were cut 4–6 h before the experiments. They were placed horizontally in Petri dishes with 2–3 ml artificial pond water (APW: 0.1 mM KCl, 0.1 mM CaCl\(_2\), 0.5 mM NaCl, unbuffered pH 5.5). *Chara* branchlet cells were chosen for use because the whole cell can be viewed in one microscope field and the cessation of cytoplasmic streaming was considered as an event when it stopped in the whole cell, not only in the location of electrodes.

#### Ion flux measurements

Ion fluxes were measured non-invasively using the MIFE system (University of Tasmania, Hobart, Australia) generally as described by Newman (2001). The ion-selective microelectrodes were backfilled by 0.15 mM NaCl and 0.4 mM KH\(_2\)PO\(_4\) (adjusted to pH 6.0 using NaOH) for hydrogen, 0.5 M NaCl for sodium, and 0.5 M KCl for potassium. The electrode tips were filled with commercial ionophore cocktails (hydrogen 95297; potassium 60031; calcium 21048, Fluka). The electrodes were calibrated in a set of standards (pH from 4.6 to 7.8; Ca\(^{2+}\) from 0.1 to 10 mM; K\(^{+}\) from 1 to 100 mM). Electrodes with responses less than 50 mV per decade for monovalent ions and 25 mV per decade for calcium were discarded.

#### Experimental procedure

The *Chara* cells were measured in the same Petri dishes placed on an inverted microscope. During measurements the cells were immobilized by a small piece of glass, which was placed on the cell wall remaining from the neighbouring internodal cell.

Electrodes were lowered into the solution and their tips were positioned near the cell in the plane of its optical section. They were moved horizontally along a cell radius between 30 \(\mu\)m and 60 \(\mu\)m from its surface using a WR888 micromanipulator (Narishige, Japan) in a square-wave manner with a 10 s cycle. Experiments were performed at 20–22 °C, under normal laboratory lighting. Fluxes of the three specific ions were measured simultaneously.

In mapping experiments each point, at 0.2–0.5 mm separations along the cell axis, was measured for 2–3 min. Since cells varied in size, about 7±1 mm, they were normalized to 7 mm, recalculating proportionally the other distances along the cell. For each cell, mean pH and fluxes over these times at each point were calculated and plotted. For each cell, correlations between pH and fluxes along the cell were calculated from those mean values (\(n=18–31\)). Means of correlation coefficients from 12 cells are shown in Table 1, with standard errors of those means. Mapping after CD and Gd\(^{3+}\) treatment was made at least 2 h after the reagent addition.

In experiments with CD (bath concentration 25 \(\mu\)M) and GdCl\(_3\) (500 \(\mu\)M), stock solutions were added to the Petri dish in several spots

### Table 1. Correlation between \(\text{H}^{+}\), \(\text{K}^{+}\), and \(\text{Ca}^{2+}\) fluxes and pH for both sides of Chara branchlets

Correlation coefficients were calculated for each cell separately. Statistics are means of coefficients from 12 cells ±SE. Levels of significance for the correlation coefficient are indicated by (*) for 5% and by (**) for 1%.

<table>
<thead>
<tr>
<th>Side A</th>
<th>pH</th>
<th>(\text{H}^{+})</th>
<th>(\text{K}^{+})</th>
<th>(\text{Ca}^{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{H}^{+})</td>
<td>0.66±0.06*</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{K}^{+})</td>
<td>0.17±0.07</td>
<td>0.16±0.09</td>
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<td></td>
</tr>
<tr>
<td>(\text{Ca}^{2+})</td>
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<td>0.22±0.09</td>
<td>0.79±0.05**</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Side B</th>
<th>pH</th>
<th>(\text{H}^{+})</th>
<th>(\text{K}^{+})</th>
<th>(\text{Ca}^{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{H}^{+})</td>
<td>0.82±0.03**</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{K}^{+})</td>
<td>0.49±0.04</td>
<td>0.58±0.04*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>(\text{Ca}^{2+})</td>
<td>0.54±0.07</td>
<td>0.70±0.03*</td>
<td>0.67±0.04*</td>
<td>1</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic diagram of *Chara australis* branchlet cells. (A) The second whorl from the top of the plant. The branchlet cell that was used in experiments is circled. (B) The side of the branchlet where cyclosis went acropetally is designated side A; where it went basipetally, side B.
in a circle close to the edge. The bath solution was mixed by sucking and expelling it from a 1 ml pipette several times. The time required for reagent addition, mixing, and establishing the diffusion gradients was discarded from the analysis and appears as a gap in Figs 3 and 5 of the Results. The CD stock solution was freshly made every day before the experiments by dissolving CD in DMSO to give a final concentration of DMSO of 0.2% (v/v) in the Petri dish. No effect of 0.2% DMSO on cyclosis and ion fluxes was observed in control experiments.

Cytoplasmic streaming was assessed under the microscope, with the chloroplasts in focus to measure the streaming rate at the site of the generation of the motive force (Staves, 1995), by counting the time required for small particles to travel 200 µm.

Results

Ion flux mapping of Chara branchlets and their nodal cells

As expected, branchlets formed alkaline and acidic bands whose pH ranged from 5 to 8. The pH profile and the K⁺, Ca²⁺, and H⁺ flux profiles along each side of three representative Chara branchlet cells are presented in Fig. 2. Different pH and ion flux patterns were observed for different sides of the branchlets. Sides with cytoplasm streaming acropetally (side A) had the greater variation in pH and H⁺ fluxes. The mean amplitude of this variation, for

![Fig. 2](https://example.com/fig2.png)

Fig. 2. Net pH profiles (acid upwards) and ion fluxes (influx positive) along each side in three representative branchlet cells. ‘N’ indicates the basal node. (A, B) pH profiles for sides A and B; (C, D) H⁺ fluxes; (E, F) Ca²⁺ fluxes; (G, H) K⁺ fluxes. Initial pH or flux values are shown at the left of each side A trace. Initial values for side B are similar.
pH and each flux in the most prominent pH band of side A, is shown in Table 2 (control).

Generally, H⁺ fluxes were consistent with H⁺ extrusion being the cause of acidic banding: at regions of lower pH greater H⁺ efflux was observed, while for alkaline areas the efflux was smaller or even shifted to influx. Thus, the correlation between pH and H⁺ fluxes was high: 0.66 for side A (P<0.05) and 0.82 for side B (P<0.01) (Table 1). For acropetal streaming (Table 1, side A) there was only small, insignificant correlation between pH or H⁺ flux and either K⁺ or Ca²⁺ fluxes. For basipetal streaming (Table 1, side B) those correlations were much higher and positive: between H⁺ and K⁺ or Ca²⁺ fluxes: 0.67 for side B (P<0.05) and 0.79 for side A (P<0.01).

The branchlet cell basal node showed two differences from the cell. (i) The node had relatively stable pH in the range 6.1 to 6.5, midway between the banding extremes for the cell. (ii) The node had a higher Ca²⁺ influx (or smaller range 6.1 to 6.5, midway between the banding extremes for side A (Fig. 2). The side A mean of the Ca²⁺ flux difference between the node and its adjacent cell is 33±4 nmol m⁻² s⁻¹ (SE, n=6). For K⁺ flux, this mean difference is 5±10 nmol m⁻² s⁻¹ (SE, n=6).

**Correspondence between ion fluxes and cytoplasmic streaming**

The application of 25 μM CD, which prevents actin from polymerizing, immediately slowed cyclosis, with inhibition complete by about 15 min (Fig. 3), in agreement with Collings et al. (1996).

H⁺ fluxes, measured at a location about 2 mm from the basal node, demonstrated a multiphasic response with an initial slight shift towards influx, subsequently returning almost to initial values (Fig. 3). After cyclosis stopped, H⁺ fluxes tended to influx. Further exposure for 2 h or more led to a significant reduction of H⁺ and pH banding patterns (Fig. 4A; Table 2).

Net K⁺ fluxes, also measured about 2 mm from the basal node, showed no significant change until an abrupt shift towards efflux happened a few minutes after cyclosis ceased (Fig. 3). Ca²⁺ fluxes demonstrated a slight initial efflux (opposite to the H⁺ flux changes) in the first 5-10 min after CD application, followed by a trend to recover the initial values. However, similar to K⁺ fluxes 5–10 min after the cessation of cyclosis in the observed area, net Ca²⁺ flux changed towards efflux. In absolute values, 2 h of cell exposure to 25 μM CD resulted in a significant reduction in magnitudes of K⁺ and Ca²⁺ flux variation within the bands (Table 2), with direction change from influx to efflux by 29 ± 4 nmol m⁻² s⁻¹ for K⁺ and 22 ± 1 for Ca²⁺ (SE, n=3) (Fig. 4B).

To help identify the process responsible for changes in pH banding, cytoplasmic streaming cessation or changes in ion transport itself, the Ca²⁺ channel blocker Gd³⁺ was used. Application of 500 μM Gd³⁺ induced enormous transient efflux of Ca²⁺ from both the node and its branchlet (Fig. 5). Following the transient (lasting 15 min for nodes and 5 min for branchlet cells) a small net Ca²⁺ efflux

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**Table 2. Magnitude of variation in pH and each ion flux in the strongest band of side A in a branchlet cell before (control) and 2 h after application of CD or Gd³⁺, or CD with Gd³⁺ pretreatment, for the same band**

The strongest band was chosen from its pH/H⁺ amplitude. Flux units, nmol m⁻² s⁻¹. Means ±SEM (n=4–12). Levels of significance for flux difference before and after treatments are indicated by ** for 1% and by **** for 0.01%.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+CD</th>
<th>+Gd³⁺</th>
<th>+CD after Gd³⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.63±0.04</td>
<td>0.08±0.01****</td>
<td>0.54±0.1</td>
<td>0.03±0.01****</td>
</tr>
<tr>
<td>H⁺</td>
<td>48.3±6.1</td>
<td>8.2±6.09****</td>
<td>33.7±6.7***</td>
<td>5.2±0.2****</td>
</tr>
<tr>
<td>K⁺</td>
<td>43.5±7.6</td>
<td>8.6±0.8***</td>
<td>12±1.3****</td>
<td>21±6.9****</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>33.7±3.9</td>
<td>5.6±0.2****</td>
<td>4.5±0.7****</td>
<td>3.6±0.2****</td>
</tr>
</tbody>
</table>

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**Fig. 3.** A representative experiment (out of 12) showing the effects of the addition of 25 μM cytochalasin D into the medium at 5 min (arrowed). Cytoplasmic streaming was recorded in the area that could be observed under the microscope close to the electrodes. Electrodes were positioned at 2 mm from the basal node of side A. Initial cyclosis speed was about 60 μm s⁻¹. Cyclosis stopped about 15 min after CD application.
continued. Two hours after Gd3+ application \((n=4)\), Ca2+ fluxes had changed towards efflux in zones where initially there was influx, or to a bigger efflux where there was already efflux (Fig. 4D). The mean change was 27 ± 3 nmol m\(^{-2}\) s\(^{-1}\) (SE, \(n=4\)) for the whole cell. Gd3+ application also caused a transient H+ efflux of similar duration to the Ca2+ transient (Fig. 5). This observation is in good agreement with earlier findings made on internodal cells of Chara corallina, where H+ currents were transiently inhibited by Gd3+ and La3+ (McConnaughey and Falk, 1991). However, 2 h pretreatment with 500 lM GdCl3 had little effect on H+ fluxes or pH (Fig. 4C) and on the amplitudes of H+ and pH profiles (Table 2). The immediate (Fig. 5) and prolonged (Fig. 4D) effect of Gd3+ on K+ fluxes was negligible, although with some small initial transient efflux from the node. During and after GdCl3 application, cyclosis was observed to be unaffected (data not shown).

The massive transient ion efflux, with a time-course of around 15 min, is consistent with the trivalent Gd3+ displacing Ca2+ (and to a lesser extent H+ and K+) from the Donnan system of the cell walls (Ryan et al., 1992). The longer term flux changes are not affected by this exchange.

For cells pretreated for 2 h in 500 μM GdCl3, a subsequent 2 h addition of 25 μM CD led to the cessation of cytoplasmic streaming (data not shown) and to a significant reduction in the magnitude of H+ fluxes (Fig. 4C). This treatment also reduced the amplitudes of the pH and H+ profiles (Table 2; Fig. 4C) in a way similar to the effect of CD on cells without Gd3+.

To summarize the results presented in Fig. 4A and C, and in Table 2, for prolonged treatments with CD and Gd3+, H+ fluxes and pH are mainly influenced by CD treatments rather than by the application of the Ca2+ channel blocker Gd3+. Ca2+ and K+ fluxes were affected by each pretreatment. These observations, with the correlation levels presented in Table 1 for cells without any treatments, indicate that K+ and Ca2+ transport in Chara cells is independent of H+ transport and pH band formation.

**Discussion**

**Ion fluxes along alkaline and acidic bands**

It has been known for 30 years that internodal cells of Chara form acid and alkaline bands during illumination (Lucas and Smith, 1973). Despite the pH band phenomenon having been confirmed in numerous studies, the mechanism of its formation and its function are still unclear. On the
other hand, transport of many ions at the boundary of the Chara internodal cell with the bathing solution has been characterized using different techniques. Radioisotopes were used in studies on Na\textsuperscript{+}, Cl\textsuperscript{−}, and Ca\textsuperscript{2+} fluxes (Hope and Walker, 1975; Findlay et al., 1969; Sanders, 1980; MacRobbie and Banfield, 1988; Hoffman et al., 1989; Whittington and Smith, 1992; Whittington and Bisson, 1994). K\textsuperscript{+} and Cl\textsuperscript{−} channels, inward and outward rectifiers were characterized in Chara using voltage and patch clamping techniques (Smith, 1984; Beilby, 1985; Tyerman et al., 1986; Coleman 1986; Kourie and Findlay, 1990; Homann and Thiel, 1994; McCulloch et al., 1997). Ion specific microelectrodes for Ca\textsuperscript{2+} were used to characterize Ca\textsuperscript{2+}/H\textsuperscript{+} exchange in the wall during algal calcification (McConnaughey, 1991; McConnaughey and Falk, 1991), and during progressively increased external concentrations of K\textsuperscript{+} and H\textsuperscript{+} (Ryan et al., 1992). However, with current knowledge, the present work is the first attempt to characterize the contribution of different ions in the overall electrical currents along the pH profiles in Chara.

From these results it is clear that OH\textsuperscript{−}/H\textsuperscript{+} fluxes contributed significantly in the formation of alkaline and acidic bands, confirming theoretical calculations made by Lucas et al. (1977). Despite an emerging literature showing the regulation of K\textsuperscript{+} transport by external or internal pH in plants (Hoth et al., 1997; Gaymard et al., 1998; Lacombe et al., 2000), these results link K\textsuperscript{+} transport with Ca\textsuperscript{2+} rather than with pH: for both sides K\textsuperscript{+} and Ca\textsuperscript{2+} fluxes are significantly correlated (Table 1). In fact, interdependence between K\textsuperscript{+} and Ca\textsuperscript{2+} transport for Chara has been described previously. Removal of Ca\textsuperscript{2+} from the medium results in the depolarization of the Chara internodal cell and an increase in membrane conductance (G\textsubscript{m}). The increase in conductance was thought to be associated with an increase in K\textsuperscript{+} conductance, as judged by Ca\textsuperscript{2+} effects on the K\textsuperscript{+} dependence of clamp current (Bisson, 1984). First of all, this interdependence could be explained by a Ca\textsuperscript{2+} effect on K\textsuperscript{+} transport: strong Ca\textsuperscript{2+} dependence of K\textsuperscript{+} channels has been shown for many higher plants and algae (Spalding et al., 1992; Plieth et al., 1998; Bauer et al., 1998). Conversely, it is possible that Ca\textsuperscript{2+} transport is regulated by K\textsuperscript{+} via changes in E\textsubscript{m}; voltage regulation of Ca\textsuperscript{2+} channels has been demonstrated in many studies (Katsuhara and Tazawa, 1992; Shimmen, 1997; Reid et al., 1997).

Interaction of ion transport with cytoplasmic streaming

Ca\textsuperscript{2+} and K\textsuperscript{+} transport: These results indicate that Ca\textsuperscript{2+} and K\textsuperscript{+} transport is closely linked with cytoplasmic streaming.
The time at which cyclosis stops in the whole cell coincides with increased \(\text{Ca}^{2+}\) influx and, probably as a consequence, the change in \(\text{K}^+\) fluxes. Dependence of ion transport on the cytoplasmic streaming rate has been described previously. In studies on ion transport across the node from one internodal cell to another, it was found that rate of intercellular \(\text{Cl}^-\) and \(\text{Rb}^+\) transport is highly dependent on the rate of cytoplasmic streaming (Bostrom and Walker, 1976; Ding and Tazawa, 1989). It has also been demonstrated that cytoplasmic streaming depends on \([\text{Ca}^{2+}]_{\text{cyt}}\) with high concentrations of \([\text{Ca}^{2+}]_{\text{cyt}}\) causing inhibition (Tominaga and Tazawa, 1981; Williamson and Ashley, 1982).

**\(\text{H}^+\) fluxes and \(\text{pH}\) band formation:** Further evidence for the interaction of cytoplasmic streaming with ion fluxes is the observed asymmetrical distribution of \(\text{pH}\) bands at the sides of the branchlet cell having opposite directions of cytoplasmic streaming, since cells were placed horizontally so gravitropically induced streaming polarity was abolished (Staves, 1997). An amplitude difference between the two sides of the internodal cell was also detected by Lucas and Nuccitelli (1980) and Bulychev et al. (2001). Unfortunately, in both papers the authors did not indicate the direction of the cyclosis, which does not allow the results to be compared.

The literature provides some evidence on the link between \(\text{pH}\) bands and the cytoskeleton, both microtubules and the actin–myosin complex, responsible for cytoplasmic streaming. Exposure of *Chara* cells to vinblastine, colchicine, or oryzalin caused a reduction in the extracellular current pattern and a shifting of it (Fisahn and Lucas, 1990). Because these agents all affect tubulin, the authors suggested that microtubules could be responsible for orchestrating the transmembrane currents. Further microscopical studies on microtubule alignments along \(\text{pH}\) bands established that microtubule number and alignment change between acidic and alkaline bands in *Chara* cells (Wasteneys and Williamson, 1992). However, these authors found that \(\text{pH}\) band formation was not prevented when microtubules were depolymerized with oryzalin, demonstrating that microtubules are not necessary for \(\text{pH}\) bands to develop in internodes. They concluded that some mechanisms for microtubule organization respond to the localized differences in membrane properties, rather than being responsible themselves for \(\text{pH}\) banding formation.

Few papers have described the link between the functioning of the actin–myosin complex and the formation of \(\text{pH}\) bands: (i) cyclosis inhibition transforms the band pattern to one of numerous small discs (Lucas and Dainty, 1977); (ii) slowing cytoplasmic streaming corresponds with narrower and more numerous bands (Yao et al., 1992); (iii) treatment of cells with cytochalasins, inhibitors of cytoplasmic streaming, caused the banding pattern to disappear (Bulychev et al., 2001). The results of this study support the last observations.

The results of this study lead to three conclusions: (i) It is proposed that \(\text{pH}\) band formation and \(\text{Gd}^{3+}\)-insensitive \(\text{Ca}^{2+}\) transport systems in *Chara* branchlets are linked with the cyclosis machinery: (a) sides with different directions of cytoplasmic streaming produce patterns with different \(\text{pH}\) amplitude range; (b) application and incubation in the \(\text{GdCl}_3\) solutions did not lead to the cessation of cytoplasmic streaming, although external \(\text{Ca}^{2+}\) fluxes changed. (ii) \(\text{K}^+\) and \(\text{Ca}^{2+}\) transport in *Chara* cells are not strongly linked with \(\text{H}^+\) transport: pre-treatment with \(\text{Gd}^{3+}\) led to changed transport properties of \(\text{K}^+\) and \(\text{Ca}^{2+}\), whereas \(\text{H}^+\) transport remained almost unaffected. (iii) There is a link between ion transport and cytoplasmic streaming: cessation of cytoplasmic streaming, some time after \(\text{CD}\) application, resulted in changed \(\text{H}^+\), \(\text{Ca}^{2+}\), and \(\text{K}^+\) fluxes and \(\text{pH}\) banding.

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**References**


