J. Membrane Biol. 114, 189–194 (1990)

The Journal of
Membrane Biology
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Topical Review

Calcium Ion and Turgor Regulation in Plant Cells

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Introduction

Turgor pressure in plant cells develops across the rigid cell wall in order to balance the osmotic pressure gradient across the plasmalemma. The maintenance of turgor pressure is indispensable in terrestrial nonlignified plant cells for providing the mechanical force to keep their form against the force of gravity. Turgor pressure is regarded as one of the factors that conceivably affects the rate of elongation growth in walled plant cells (Ray, Green & Cleland, 1972) and is involved in stomatal movements or in leaf movements of sensitive and carnivorous plants. Aquatic walled plant cells living in intertidal zones or estuaries are exposed to fluctuations of external osmotic pressure. Without turgor regulation they lose stiffness under high salinity and may suffer mechanical injuries by surf. Inversely, when salinity is too low they may be ruptured by high turgor pressure. Thus a mechanism maintaining turgor pressure to a definite value (turgor regulation) will be advantageous to growing walled plant cells, which are forced to decrease cellular osmotic pressure due to cell enlargements and are exposed to fluctuations in external osmotic pressure.

Turgor regulation has been well studied and characterized in marine algal cells because (i) many show distinct turgor regulation and (ii) they are relatively large so that ion fluxes, membrane potentials and membrane conductances are easy to measure (for reviews, *see* Cram, 1976; Hellebust, 1976; Gutknecht, Hastings & Bisson, 1978; Kauss, 1978; Zimmermann, 1978; Kirst & Bisson, 1979; Munns, Greenway & Kirst, 1983). In this article, we focus our attention on the turgor regulation controlled by Ca^{2+} in algal cells (*Lamprothamnium* and *Pelvetia*). Due to limitations in space, Ca^{2+}-controlled phenomena other than turgor regulation will not be covered in this review. For reviews of these related phenomena, the readers should consult papers by Marmé (1983), Dieter (1984), Macklon (1984), Hepler and Wayne (1985), Trewavas (1985) and Gilroy, Blowers and Trewavas (1987), and Kauss (1987).

Key Words algae · calcium · ion transport · plant cell · turgor pressure · turgor regulation

Lamprothamnium Cells Regulate Turgor Pressure Upon Hypo- and Hypertonic Treatments

Lamprothamnium is one of the genera of euryhaline characean algae. Internodal cells of this alga are cylindrical giant cells whose volumes are ca. several mm^3. They can maintain their turgor pressure to a nearly constant value (ca. 0.8 MPa) against changes in the external osmotic pressure. By contrast, internodal cells of the fresh water Characeae regulate not their turgor pressure but their cellular osmotic pressure (*see* review of Tazawa, Shimmen & Mimura, 1987). The turgor regulation in *Lamprothamnium* was studied on ion transports with two species, *L. papulosum* (Bisson & Kirst, 1980*a,b*; Jefferies & Reid, 1984; Reid, Jefferies & Pitman, 1984; Wichmann & Kirst, 1989) and *L. succinctum* (Okazaki, Shimmen & Tazawa, 1984*a,b*).

Upon hypotonic treatment, *Lamprothamnium* cells lower the elevated turgor pressure nearly to the original level very rapidly by passively releasing K^+ and Cl^- from the vacuole (hypotonic turgor regulation). The membrane conductance transiently increases about 10-fold, attaining a peak value 2–4 min after the initiation of hypotonic treatment, and then returns to the original low value within 30–60 min. The membrane potential, which is actually the potential of the vacuole relative to that of the medium, is normally ca. −170 mV. It depolarizes promptly upon hypotonic treatment. Interestingly, the depolarized state continues for more than 60 min after completion of turgor regulation.

Upon hypertonic treatment, *Lamprothamnium*

cells regulate their turgor pressure more slowly by absorbing K^+ and Cl^- into the vacuole (hypertonic turgor regulation). The hypertonic turgor regulation is accompanied by a membrane hyperpolarization, which may be caused by the activation of the plasmalemma H^+ pump (Okazaki et al., 1984*b*).

Hypotonic Turgor Regulation Needs External Ca^{2+}

The involvement of Ca^{2+} in turgor regulation was suggested in *L. succinctum* by the fact that cells transferred into a hypotonic medium of low external Ca^{2+} concentration ($[Ca^{2+}]_e$, 0.01 mol/m^3) cannot lower the elevated turgor pressure (Okazaki & Tazawa, 1986*a*). Under the low $[Ca^{2+}]_e$, the transient increase in the membrane conductance is suppressed while the membrane depolarization is not inhibited.

We were unable to test the contribution of external Ca^{2+} to hypertonic turgor regulation, since the removal of Ca^{2+} for the protracted periods necessary for hypertonic turgor regulation results in cell death.

Cytoplasmic Free Ca^{2+} Concentration Increases during Hypotonic Turgor Regulation

In order to study the correlation between hypotonic turgor regulation and cytoplasmic Ca^{2+}, we measured both the cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_c$) and total cytoplasmic calcium content. The $[Ca^{2+}]_c$ was measured with a Ca^{2+}-sensitive photoprotein, aequorin, which was injected into the cytoplasm (Okazaki et al., 1987). The total cytoplasmic calcium content was measured by atomic absorption spectrophotometry using internodal cells whose vacuoles had been perfused with Ca^{2+}-free artificial cell saps (Okazaki & Tazawa, 1987*a*).

The light emission of injected aequorin ($[Ca^{2+}]_c$) remains low when the cells are at a steady-state turgor pressure. However, in response to hypotonic treatment, the light emission increases with a time lag of ca. 1 min. It reaches a peak value about 2–4 min after the treatment and then recovers to the original low level within 30 min. The kinetics of aequorin light emission correlates with those of the hypotonic turgor regulation, which completes within approximately 40 min (Okazaki et al., 1984*b*). The aequorin light emission, which occurs as a result of hypotonic treatment, is completely inhibited by lowering $[Ca^{2+}]_e$ from 3.9 to 0.01 mol/m^3 (Okazaki et al., 1987).

The total cytoplasmic calcium content in *Lamprothamnium* at the steady-state turgor pressure is ca. 1–2 mol/m^3. Upon hypotonic treatment, it increases by 1–2 mol/m^3 after 1 hr. The increase in the total calcium content does not occur under low $[Ca^{2+}]_e$. The fact that the total cytoplasmic calcium content is much higher than the $[Ca^{2+}]_c$ suggests that cytoplasmic calcium is stored either as the bound form or in cell organelles, including the mitochondria, the endoplasmic reticulum and the chloroplasts.

An Increase in Turgor Pressure may Activate the Plasmalemma Ca^{2+} Channel

The fact that lowering $[Ca^{2+}]_c$ inhibits the increases in both the ionized and total calcium content in the cytoplasm indicates that the Ca^{2+} influx is enhanced upon hypotonic treatment. Inhibition of the turgor regulation by a Ca^{2+} channel blocker, nifedipine, suggests that the entry of external Ca^{2+} is mediated by Ca^{2+} channels (Okazaki & Tazawa, 1986*b*).

The Ca^{2+} channels in *Lamprothamnium* do not seem to be voltage dependent, since the membrane depolarization induced by the hypotonic treatment continues after the recovery of turgor pressure. However, it is possible that the Ca^{2+} channel opens in response to membrane depolarization and that the activated Ca^{2+} channel is then inactivated in a voltage-independent manner.

A mechanical activation of the Ca^{2+} channel is known to take place in *Paramecium* whose plasmalemmal Ca^{2+} channel is activated by a mechanical deformation (Naitoh & Eckert, 1969) and in the choroid plexus epithelium whose stretch-activated ion channels may function as a Ca^{2+} channel. The latter channel is activated by hypotonic treatment (Christensen, 1987).

Ca^{2+} Channel Exists in Plant Cells

The existence of Ca^{2+} channels has been inferred in higher plant cells (Hetherington & Trewavas, 1984; Andrejauskas, Hertel & Marmé, 1985; Hepler & Wayne, 1985 (review); Graziana et al., 1988) and fresh water Characeae cells (Tsutsui et al., 1987; MacRobbie & Banfield, 1988).

The presence of a plasmalemmal Ca^{2+} channel was conclusively demonstrated by Shiina and Tazawa (1987*a*) in characean cells (*Nitellopsis obtusa*) cultivated in fresh water. They prepared tonoplast-free cells by perfusing the vacuole with a salt solution containing ethyleneglycol-bis-(β-aminoethyl ether)N,N,N′,N′-tetraacetic acid (EGTA), which degrades the tonoplast by chelating Ca^{2+} (Ta-

zawa, Kikuyama & Shimmen, 1976). Due to a very low intracellular Ca^{2+} concentration ($[Ca^{2-}]_i$) in tonoplast-free cells, the plasmalemma Cl^- channel, which is activated in normal cells by electrical stimulations, cannot be activated. Then the inward current measured under the voltage-clamp condition was found to be carried by only Ca^{2+}. The current was not inhibited by anion channel blockers but by Ca^{2+} channel blockers.

We are not sure whether or not similar Ca^{2+} channels are present in the plasmalemma of *Lamprothamnium* of the brackish water Characeae. The Ca^{2+} channel in *Nitellopsis* is voltage dependent, but the Ca^{2+} channel in *Lamprothamnium* may not be voltage dependent but rather controlled directly by turgor pressure. The problem should be solved by conducting experiments, including voltage-clamping tonoplast-free *Lamprothamnium* cells.

Plasmalemma and Tonoplast Ion Channels in *Lamprothamnium* may be Activated by Ca^{2+}

In order to study whether or not K^+ and Cl^- effluxes are triggered by an increase in $[Ca^{2+}]_c$ in *Lamprothamnium*, we prepared tonoplast-free cells having various free $[Ca^{2+}]_i$ (Okazaki & Tazawa, 1987*b*). Membrane conductance of the plasmalemma in these cells does not depend on $[Ca^{2+}]_i$. By contrast, the plasmalemma potential of these cells depolarizes at high $[Ca^{2+}]_i$ (10^{-2} mol/m^3).

Electrophysiological studies of the tonoplast were conducted using cytoplasmic drops isolated from internodal cells. The surface of the drop originates from the tonoplast (Sakano & Tazawa, 1986; Lühring, 1986). Patch-clamp studies on the surface membranes of the cytoplasmic drop show that the tonoplast of *Lamprothamnium* has Ca^{2+}-activated K^+ channels whose unitary conductance is large enough to account for the net K^+ efflux during hypotonic turgor regulation (Katsuhara, Mimura & Tazawa, 1989).

These results show that an increase in $[Ca^{2+}]_c$ activates the plasmalemma K^+ channel and/or Cl^- channel not directly but indirectly through soluble Ca^{2+}-sensitizing components, which may be lost in cells in which the tonoplast has been removed. By contrast, the tonoplast K^+ channel may be activated directly by an increase in $[Ca^{2+}]_c$.

Ca^{2+}-Activated Anion Channels Exist in Plant Cells

The Ca^{2+}-activated anion channels are present in the plasmalemma of fresh water Characeae cells (Findlay Hope, 1964; Lunevsky et al., 1983; Tazawa et al., 1987 (review); Tsutsui et al., 1987) and of a water mold, *Blastocladiella emersonii* (Caldwell, Brunt & Harold, 1986). Patch-clamp studies on the plasmalemma of guard cells of *Vicia faba* showed that elevated $[Ca^{2+}]_c$ activated a voltage-dependent depolarizing conductance with a permeability to anions (Schroeder & Hagiwara, 1989).

The existence of a Ca^{2+}-activated Cl^- channel in the plasmalemma of a fresh water Characeae *Nitellopsis*, was demonstrated by Shiina and Tazawa (1987*b*, 1988). Both the inward current measured under voltage clamping and the Cl^- efflux are reduced by the Cl^- channel inhibitor 9-anthracenecarboxylic acid (A-9-C) and by La^{3+}. The Cl^- efflux is also greatly reduced by decreasing $[Ca^{2+}]_c$ (Shiina & Tazawa, 1987*b*). Direct manipulation of $[Ca^{2+}]_i$ in tonoplast-free cells indicates that the large Cl^- efflux and the concomitant membrane depolarization are caused by an increase in $[Ca^{2+}]_i$ (more than 4×10^{-3} mol/m^3). The Ca^{2+}-activated Cl^- channel is also present in the tonoplast of a fresh water Characeae, *Chara corallina* (Kikuyama, 1988).

A Model of Hypotonic Turgor Regulation in *Lamprothamnium*

We propose the following model for the process of hypotonic turgor regulation (Fig.). First, the Ca^{2+} channel is activated by an increase in turgor pressure. This Ca^{2+} channel activation seems to be independent of Ca^{2+} influx, since it occurs under the low $[Ca^{2+}]_e$ (0.01 mol/m^3) (Okazaki & Tazawa, 1986*a*; Okazaki et al., 1987). The increase in $[Ca^{2+}]_c$ caused by the enhanced Ca^{2+} influx down the electrochemical potential gradient activates K^+ and/or Cl^- channels in the plasmalemma and/or the tonoplast. Passive effluxes of K^+ and Cl^- from both the cytoplasm and the vacuole to the external medium ensue until the Ca^{2+} channel is inactivated. The inactivation occurs upon the recovery of the turgor pressure to the original level.

Entry of External Ca^{2+} Induces Cl^- Net Efflux during Turgor Regulation in *Pelvetia*

Eggs of *Pelvetia fastigiata* (a brown alga belonging to Fucaceae) form cell walls 3–4 hours after fertilization and subsequently develop turgor pressure through absorption of K^+ and Cl^- (Allen et al., 1972). Unilateral illumination of fertilized eggs initiates embryogenesis with the formation of rhizoid on the shaded side. After illumination, a localized Ca^{2+}

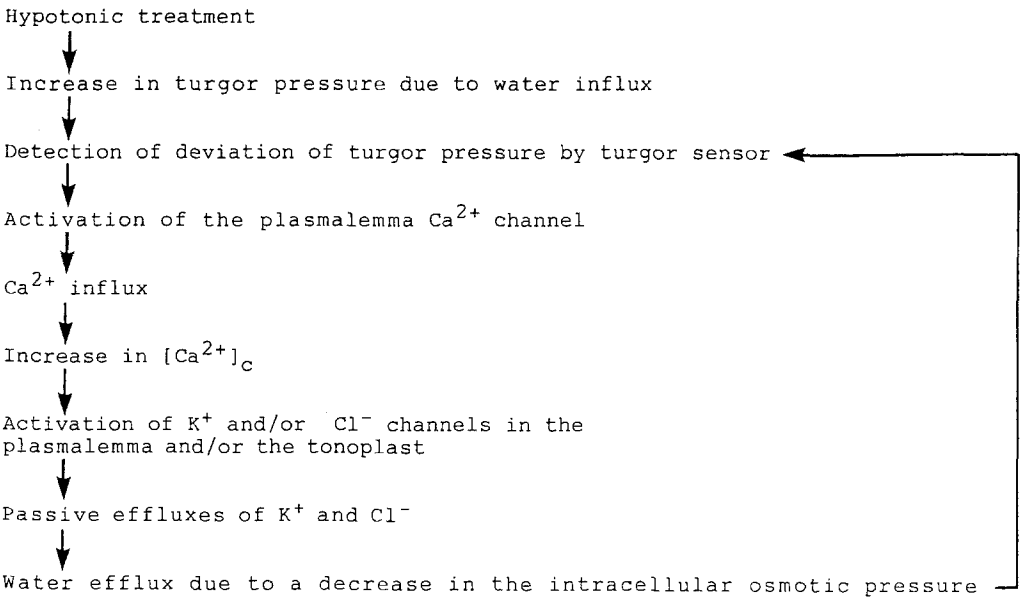

Fig. The scheme of hypotonic turgor regulation in *Lamprothamnium*

influx into the rhizoidal tip occurs (Robinson & Jaffe, 1975; Robinson & Cone, 1980). The localized Ca^{2+} influx causes the localization of membrane-associated Ca^{2+} (Kropf & Quatrano, 1987). The intracellular gradient of $[Ca^{2+}]_c$ is shown in *Fucus* (another species of Fucaceae) (Brownlee & Wood, 1986; Brownlee & Pulsford, 1988).

Soon after germination, occasional spontaneous current pulses are observed with a vibrating electrode. The current pulses enter the growing rhizoidal tip and leave the rest of the embryo (Nuccitelli & Jaffe, 1974). The inward pulse current is carried mainly by Cl^- (Cl^- efflux), and the outward pulse current is carried by K^+ (K^+ efflux). These pulses are stimulated or suppressed by a decrease or increase in the external osmotic pressure (Nuccitelli & Jaffe, 1976*a*). The rate of $^{36}Cl^-$ efflux increases during the first 10 min after lowering of the osmotic pressure (hypotonic treatment). The efflux rate returns to the original low value 30 min after hypotonic treatment. If the rate of K^+ efflux is equivalent to the rate of Cl^- efflux, then the resulting changes in the intracellular osmotic pressure are sufficient to account for the recovery of the turgor pressure (Nuccitelli & Jaffe, 1976*b*). Nuccitelli and Jaffe (1976*a,b*) proposed the following scheme: Elevation of turgor pressure activates the plasmalemma Ca^{2+} channels by stretching the plasmalemma at the tip; the enhanced Ca^{2+} influx results in an increase in $[Ca^{2+}]_c$, which then activates both Cl^- channels and K^+ channels. The resultant K^+ and Cl^- effluxes relieve the excess turgor pressure.

Cytoplasmic Free Ca^{2+} may Act as a Controlling Signal in Negative Feedback Turgor Regulation

Turgor regulation is postulated to depend upon a negative feedback system (Cram, 1976; Gutknecht et al., 1978). The putative turgor-sensing mechanism detects the deviation of turgor pressure from a reference value (an error signal) and transduces it into a controlling signal. This controlling signal then controls the passive and active ion transport activities. The change in the cellular osmotic pressure that results from the changes in the ion concentrations leads to changes in the turgor pressure. This information is again detected by the turgor-sensing mechanism and the whole process begins again (for more detail, *see* Gutknecht et al., 1978; Kauss, 1978; Zimmermann, 1978).

In the case of turgor regulation in *Lamprothamnium* and *Pelvetia*, the error signal is the elevation of the turgor pressure and the controlling signal is an increase in $[Ca^{2+}]_c$. This increase in $[Ca^{2+}]_c$ results from an enhanced Ca^{2+} influx from the external medium through plasmalemma Ca^{2+} channels. The increased $[Ca^{2+}]_c$ activates K^+ and/or Cl^- channels in the plasmalemma and/or the tonoplast.

Ca^{2+} is Involved in Turgor-Driven Movements in Higher Plants

Leaf movements in sensitive plants (Sibaoka, 1969), nyctinastic plants (Satter, 1979; Satter & Galston,

1981) and carnivorous plants (Bentrup, 1979) are driven by changes in the turgor pressure of motor cells. Leaf closure results from the loss of turgor pressure in motor cells.

In a sensitive plant, *Mimosa*, the turgor reduction accompanies action potentials (Cl^- spike) (Samejima & Sibaoka, 1982) and transient large net effluxes of K^+ and Cl^- (Samejima & Sibaoka, 1980; Abe, 1981; Kumon & Suda, 1984; Kumon & Tsurumi, 1984). The leaf closure caused by the reduction in the turgor pressure of the motor cells is inhibited by ethylenediaminetetraacetic acid (EDTA) and La^{3+} (Vanden Driessche, 1963; Campbell & Thomson, 1977). In carnivorous plants *Aldrovanda* and *Dionaea*, the turgor reduction is triggered by an action potential (Ca^{2+} spike), which is inhibited by La^{3+} and EGTA (Iijima & Sibaoka, 1985; Hodick & Sievers, 1986, 1988). It is not yet known how Ca^{2+} is involved in the turgor reduction of these motor cells. Calcium-sensitive contractile ATPase is postulated to be involved in releasing vacuolar content in *Mimosa* (Toriyama & Jaffe, 1972; Vanden Driessche, 1978). Hodick and Sievers (1988) proposed that a rise in $[Ca^{2+}]_c$ induced by the Ca^{2+} spike triggers the reduction of turgor pressure. It is probable that Ca^{2+} entry through voltage-gated Ca^{2+} channels activates plasmalemma anion channels in the motor cells of these plants. Lanthanum inhibition supports this hypothesis.

The opening or closing of stomata is performed by changing the turgor pressure of the guard cells, which is regulated by K^+ and its counter ions (Raschke, 1975, 1979; Hsiao, 1976; Outlaw, 1983; Zeiger, 1983). The dark-induced stomatal closure in *Commelina* is accelerated by external Ca^{2+} and inhibited by EDTA (Fujino, 1967) or EGTA (Schwartz, 1985). Schroeder and Hagiwara (1989) suggested that the membrane depolarization caused by the activation of a Ca^{2+}-activated anion channel activates an outwardly rectifying K^+ channel.

Concluding Remarks

Turgor pressure in many plant cells is regulated by passive and active ion transport. The turgor regulation in algal cells discussed in the present article suggests that passive ion transport is controlled by ion-specific channels, which are activated by $[Ca^{2+}]_c$. An increase in turgor pressure may open the Ca^{2+} channels, and then the resultant increase in $[Ca^{2+}]_c$ may regulate passive ion movements. The Ca^{2+}-induced activation of ion channels is a reasonable mechanism for the rapid reduction of turgor pressure. For the moment we cannot conclude that this sequence of ionic events is ubiquitous in hypotonic turgor regulation in plant cells.

Calcium ions seem to be involved also in the volume regulation of a wall-less unicellular alga *Poterioochromonas*. When this alga is exposed to hypertonic solutions, the cell shrinks. For the recovery of cell volume, an increase in $[Ca^{2+}]_c$ may be the controlling signal that activates isofloridoside biosynthesis in a calmodulin-dependent manner (Kauss, 1987).

It is reasonable that other plants have similar ionic mechanisms of turgor regulation. We know of many cases where turgor pressure influences ion transport (Cram, 1976). In *Valonia*, turgor pressure directly controls both active K^+ influx (Gutknecht, 1968) and the membrane conductance (Zimmerman & Steudle, 1974). Passive Cl^- release and active Cl^- uptake is regulated by turgor pressure in the green algae, *Acetabularia* (Wendler, Zimmermann & Bentrup, 1983) and *Codium* (Bisson & Gutknecht, 1977). Stretch-activated ion channels exist in walled organisms (Falke et al., 1988; Martinac et al., 1987; Gustin et al., 1988; Schroeder & Hedrich, 1989). Whether the control of ion transport by turgor pressure cited above is direct or also mediated by a Ca^{2+} signal as in *Lamprothamnium* remains to be studied.

We thank Dr. T. Shiina, Mr. M. Katsuhara and Dr. R. Wayne for critical reading of the manuscript and Dr. R. Wayne, for his help with the English text.

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Received 28 June 1989; revised 22 September 1989