Plant Physiol. (1995) 108: 897–901

Aluminum Induces Rigor within the Actin Network of Soybean Cells

Sharon Grabski and Melvin Schindler*

Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824

Aluminum is toxic to both plants and animals. Root growth and pollen-tube extension are inhibited after aluminum stress in acidic environments. Incubation of cultured neurons with aluminum results in the formation of neurofibrillar tangles reminiscent of the neural pathology observed in Alzheimer's disease. The present communication demonstrates that aluminum induces a rapid and dramatic increase in the rigidity of the actin network in soybean (*Glycine max*) root cells. This rigidity can be prevented by either co-incubation with sodium fluoride or magnesium, or pretreatment with cytochalasin D. It is proposed that the growth-inhibitory activity and cytotoxicity of aluminum in plants may be a consequence of a global rigor that is induced within the actin network. This rigor may result from the formation of nonhydrolyzable [Al^{3+}-ADP] or [Al^{3+}-ATP] complexes whose binding to actin/myosin can modify contraction. Additionally, Al^{3+}-mediated interference with the normal kinetics of F-actin filament assembly/disassembly could precipitate subsequent disorganization of associated cytoskeletal structures and promote altered expression of cytoskeletal proteins.

Aluminum (Al^{3+}) that is leached from mineral deposits by acid rain or acid-forming nitrogenous fertilizers has been demonstrated to be a significant inhibitor of plant growth in acid soils (Foy et al., 1978) and a toxicant to fish inhabiting acidified aquatic systems (Driscoll et al., 1980). Aluminum has also been implicated as a causative agent in an array of human diseases that include dialysis encephalopathy (Alfrey et al., 1972), amyotrophic lateral sclerosis/parkinsonian dementia (Garruto et al., 1990), osteomalacia (Simpson et al., 1976), and a severe microcytic hypochromic anemia (Short et al., 1980) that develops in dialysis patients exposed to aluminum overload. Although controversial, correlative evidence supports a role for aluminum in the development of β-amyloid plaques, neurofibrillary tangles, and the dementia associated with the progression of Alzheimer's disease (Perl and Brody, 1980; McLachlan et al., 1992). The abundance of aluminum products (Schenk et al., 1989; Greger, 1992) in the environment and aluminum's potential as a plant growth inhibitor and neurotoxic agent makes an understanding of the mechanism(s) of aluminum intoxication essential.

Previous work has shown that the toxicity of aluminum in both plant and animal cells may be a consequence of adverse effects on a variety of cellular pathways. Aluminum can inhibit cation transport across the plasma membrane (Koenig and Jope, 1987; Huang et al., 1992), interfere in calcium binding to calmodulin (Siegel and Haug, 1983), inhibit hexokinase (Viola et al., 1980), block K^+ uptake in root hairs (Gassmann and Schroeder, 1994), and affect cell wall biosynthesis (LeVan et al., 1994). The observations that Al^{3+} can bind to nucleoside triphosphates approximately 10^7 times better than Mg^{2+} and that the rates of hydrolysis for [Al^{3+}-ATP] or [Al^{3+}-GTP] complexes are considerably slower than for the physiological Mg^{2+} complexes (10^5 times slower) have provided support for the hypothesis that toxicity is a result of Al^{3+} displacement of Mg^{2+} from nucleoside di- or triphosphate complexes (Macdonald et al., 1987; Macdonald and Martin, 1988; Martin, 1992). Since actin and tubulin dynamics and microfilament and microtubule stability/instability are dependent on the binding and hydrolysis of [Mg^{2+}-ATP] and [Mg^{2+}-GTP] complexes, respectively, a change in the kinetics of these reactions could lead to disruptive influences on the organization of the cytoskeleton network with immediate consequences for growth-related activity, e.g. cell division and pollen-tube extension. A prediction of this hypothesis for cell function is that the cytoskeleton and, potentially, the expression of cytoskeletal proteins are primary cellular targets of aluminum toxicity. The present communication demonstrates that aluminum can initiate cellular processes leading to a significant increase in the tension within the transvacuolar actin network of soybean (*Glycine max*) root cells.

MATERIALS AND METHODS

CODA

CODA was performed as described by Grabski et al. (1994) and Schindler (1995). Briefly, a soybean (*Glycine max* [L.] Merr. cv Mandarin) cell suspension (originally derived from roots) in 1B5C media was placed on a slide to which a coverslip was applied and then sealed with paraffin wax. All effector reagents were incubated with the cells, at the concentrations indicated, for 30 min before measurement and were maintained with the cells throughout the CODA. An ACAS 570 Fluorescence Interactive Laser Cytometer (Meridian Instruments, Okemos, MI) was utilized for all CODA experiments (Grabski et al., 1994; Schindler, 1995). An argon ion laser beam was focused onto the transvacuolar strand or an associated vesicle. The intensity of the

* Corresponding author; e-mail 22345mss@ibm.cl.msu.edu; fax 1–517–353–9334.

Abbreviation: CODA, cell optical displacement assay.

trapping laser beam was then monotonically increased to a level that can maintain the optical trapping of the fiber at its initial position as the stage is moved through a defined displacement at a constant velocity. Measurements were performed in 20 different cells. The maximum trapping intensity to achieve success in all 20 displacement attempts did not vary from day to day by more than 5 mW, as recorded at the laser head (12% variation). To ensure that the trapping intensity did not damage the fibers, each displacement at a particular power setting was performed five times, and in all instances the fiber was required to rebound to the original position after termination of the trap. All trapping experiments were recorded on videotape, and individual pictures of the experiments were prepared frame by frame from the tapes.

Confocal Microscopy

Cells were permeabilized and stained with Bodipy-phallacidin (F-actin-specific fluorescent probe) (Molecular Probes, Eugene, OR) as described (Grabski et al., 1994). A total of 25 sections (at 0.5-μm intervals) were accumulated and examined. An InSight Bilateral Laser Scanning Confocal Microscope (Meridian Instruments) was utilized as described (Grabski et al., 1994).

RESULTS

Measurements of Actin Tension in Soybean Root Cells in the Presence of Aluminum

To characterize the effect of aluminum on the cytoskeletal network, we have utilized a new assay to measure the tension within the actin network of living cells. The CODA was developed to provide a quantitative tool that is capable of measuring the effect of potential cytoskeletal perturbants and hormones on the actin network in living plant cells (Grabski et al., 1994; Schindler, 1995). An optical trap is utilized to displace an actin strand in soybean root cells. Changes in the power of the trapping laser beam necessary to displace these strands through a defined distance at a constant velocity in a number of cells can be correlated to changes in tension within the actin network (Grabski et al., 1994; Schindler, 1995). An advantage of utilizing soybean root cells in suspension culture to assay for the biological activity of aluminum is that the cells are normally grown in media at pH 5.5 to 6.0. Under these conditions, a considerable fraction of the aluminum exists as the octahedral hexahydrate, $Al(H_2O)_6^{3+}$, or free Al^{3+}, the cytotoxic form of the metal (Macdonald and Martin, 1988; Martin, 1992).

As observed in Figure 1 and Table I, the addition of aluminum to soybean root cells results in a rapid and dose-dependent increase in the rigidity of the actin network. Pretreatment of cells with cytochalasin D (Grabski et al., 1994), which disrupts the integrity of F-actin filaments, prevents the rigidification induced by aluminum (Table I). This occurs to a lesser extent for amiprophosmethyl (Grabski et al., 1994), an herbicide that disrupts microtubules (Table I). These results provide evidence that the integrity of the F-actin network and, to a lesser extent, an assembled microtubule network is essential for the induction of rigor by aluminum.

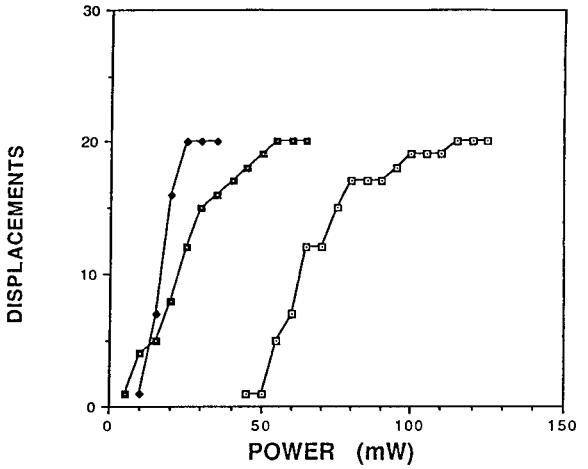

Figure 1. The effect of aluminum on the tension in transvacuolar strands in soybean root cells. The curves represent the following incubation conditions: control (♦), $AlCl_3$ (10 μM) (⊡), and $AlCl_3$ (10 μM) + NaF (10 mM) (■).

The increase in rigidity is reversible after washes with media that did not contain aluminum (Table I). This reversal after washing indicates that rigidity results from an equilibrium process rather than from aluminum-catalyzed cross-linking of proteins (Table I). Both fluoride and magnesium were shown to minimize or prevent rigidification by aluminum in a dose-dependent manner (Fig. 1; Table II). These results are consistent with previous measurements demonstrating that both magnesium and fluoride could independently prevent the inhibitory activity of aluminum toxicity for pollen tube growth (Konishi and Miyamoto, 1983) and root elongation in newly germinated red clover (Kinraide et al., 1985) and also enhance the survival of fish exposed to soluble aluminum species in acidified aquatic environments (Driscoll et al., 1980).

Confocal Fluorescence Images of the Organization of F-Actin in Soybean Root Cells

Confocal fluorescence imaging of the F-actin distribution in the presence and absence of $AlCl_3$ was performed with the F-actin probe Bodipy-phallacidin, as previously described for *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-phallacidin (Grabski et al., 1994) (Fig. 2). Optical sections obtained from fixed and labeled cells were displayed at 0.5-μm intervals. It appears that the addition of aluminum to soybean root cells (Fig. 2A) did not dramatically alter the general pattern of F-actin staining when compared to controls (Fig. 2B). White strands in both samples represent thick F-actin filaments. These images are representative of data obtained from many samples. This suggests that the changes in rigidity are probably related to physical changes in the tension of the F-actin network rather than significant changes in the amount of F-actin or its intracellular distribution.

Table I. *Aluminum-induced rigidity of the actin network in soybean root cells*

The near-linear response observed for each displacement experiment (Fig. 1; Grabski et al., 1994) suggests the possibility of utilizing the 50% point (10 successful displacements/20 attempts) (DT_{50}) for comparisons of filament tension between different experiments.

Al^{3+}	DT_{50}
μM	*mW*
Control (1B5C media)	15
0.1	15
0.5	25
1.0	30
5.0	55
10.0	65
20.0	160
50.0	170
10.0 + cytochalasin D (20 μM)	<10
10.0 + amiprophosmethyl (20 μg/mL)	40
10.0 + wash with Al^{3+}-free medium (3×) and maintain Al^{3+} free during CODA	15

Measurements of Actin Tension in the Presence of EGTA, Ba^{2+}, and Verapamil

It has previously been proposed that aluminum toxicity results from an aluminum-mediated blocking of calcium uptake (Huang et al., 1992). However, we have demonstrated that increasing the free calcium concentration in the cytoplasm of soybean root cells results in a significant increase in tension within the actin network (Grabski et al., 1994). Consequently, inhibition of calcium uptake should result in either no change or a decrease in tension. Since aluminum increases the tension in the actin network (Fig. 1; Table I), it would appear that the aluminum-mediated activity cannot occur as a result of inhibition of calcium uptake. To further examine this question, we measured the effects of EGTA (a calcium chelator), Ba^{2+} (an effective competitor of calcium uptake [Thuleau et al., 1994]), and verapamil (a Ca^{2+} channel blocker [Pantoja et al., 1992]) in the CODA. All of these agents have different mechanisms for interfering with the cellular uptake of calcium. As shown in Table III, these agents have no effect on the actin tension, providing further support that inhibition of calcium uptake is not the mechanism by which aluminum induces rigor in the actin network.

DISCUSSION

Evidence has been presented in this communication to propose that a major response of plant cells to aluminum is an increase in the tension within the actin network. Previous measurements have suggested that aluminum can also interfere with cation fluxes (Huang et al., 1992; Gassmann and Schroeder, 1994), in particular Ca^{2+} (Huang et al., 1992), across the plasma membrane. In a recent study by Ryan et al. (1994), it was observed that low concentrations of aluminum incubated with wheat seedlings could inhibit root growth without inhibiting Ca^{2+} uptake, and that the addition of other cations (e.g. Na^{+}, Mg^{2+}) to aluminum-treated roots improves root growth, while at the same time inhibiting Ca^{2+} uptake. They conclude that the inhibition of root growth by low concentrations of aluminum appears to be caused by other interactions rather than by an inhibition of Ca^{2+} uptake. In work by Gassmann and Schroeder (1994), it was concluded that inhibition of K^{+} fluxes in channels in root hairs by aluminum is not likely to be involved in mediating rapid inhibition of immature seedling growth. These two observations, in conjunction with our results showing both a positive effect of free Ca^{2+} on tension (Grabski et al., 1994) and the lack of an effect on tension after incubation with inhibitors of Ca^{2+} uptake (Table III), would appear to rule out a role for aluminum-mediated modifications of ionic fluxes in the tension-inducing activity of aluminum and, presumably, also in the growth-inhibition activity. In contrast, the aluminum-mediated induction of rigor within the actin network would have an immediate impact on the growth of root cells.

An alternative mechanism for the influence of aluminum on actin tension may occur through the replacement of Mg^{2+} by Al^{3+} in [metal ion-ATP] complexes (Macdonald et al., 1987; Macdonald and Martin, 1988; Martin, 1992). In vitro measurements of [Al^{3+}-GTP-microtubule] complexes demonstrated that such complexes were considerably more stable and less sensitive to calcium depolymerization (Macdonald et al., 1987). In examining the means by which [Al^{3+}-ATP] complexes could compromise the normal patterns of F-actin assembly/disassembly, it may be relevant to examine the mechanism proposed for azide-induced stiffness, as demonstrated in lymphocytes (Pasternak and Elson, 1985; Pasternak et al., 1989) and soybean root cells (Grabski et al., 1994). It has been speculated that the stiffening of the actin network in lymphocytes (Pasternak and Elson, 1985; Pasternak et al., 1989) and soybean root cells (Grabski et al., 1994) occurs as a result of a contraction in the actin/myosin network that is analogous to rigor contraction induced by ATP depletion in muscle (Pasternak and Elson, 1985; Pasternak et al., 1989). The formation of nonhydrolyzable [Al^{3+}-ATP] could lead to a similar contraction of actin/myosin by effectively depleting the concentration of hydrolyzable ATP and also by decreasing the

Table II. *Protective effect of NaF and Mg^{2+} on aluminum-induced tension*

DT_{50}, Fifty percent point (10 successful displacements/20 attempts).

Al^{3+} (10 μM)	DT_{50}
	mW
+NaF (*x* mM)	
0.0	65
2.0	25
5.0	20
20.0	15
+Mg^{2+} (*x* mM)	
2.0	15
5.0	15

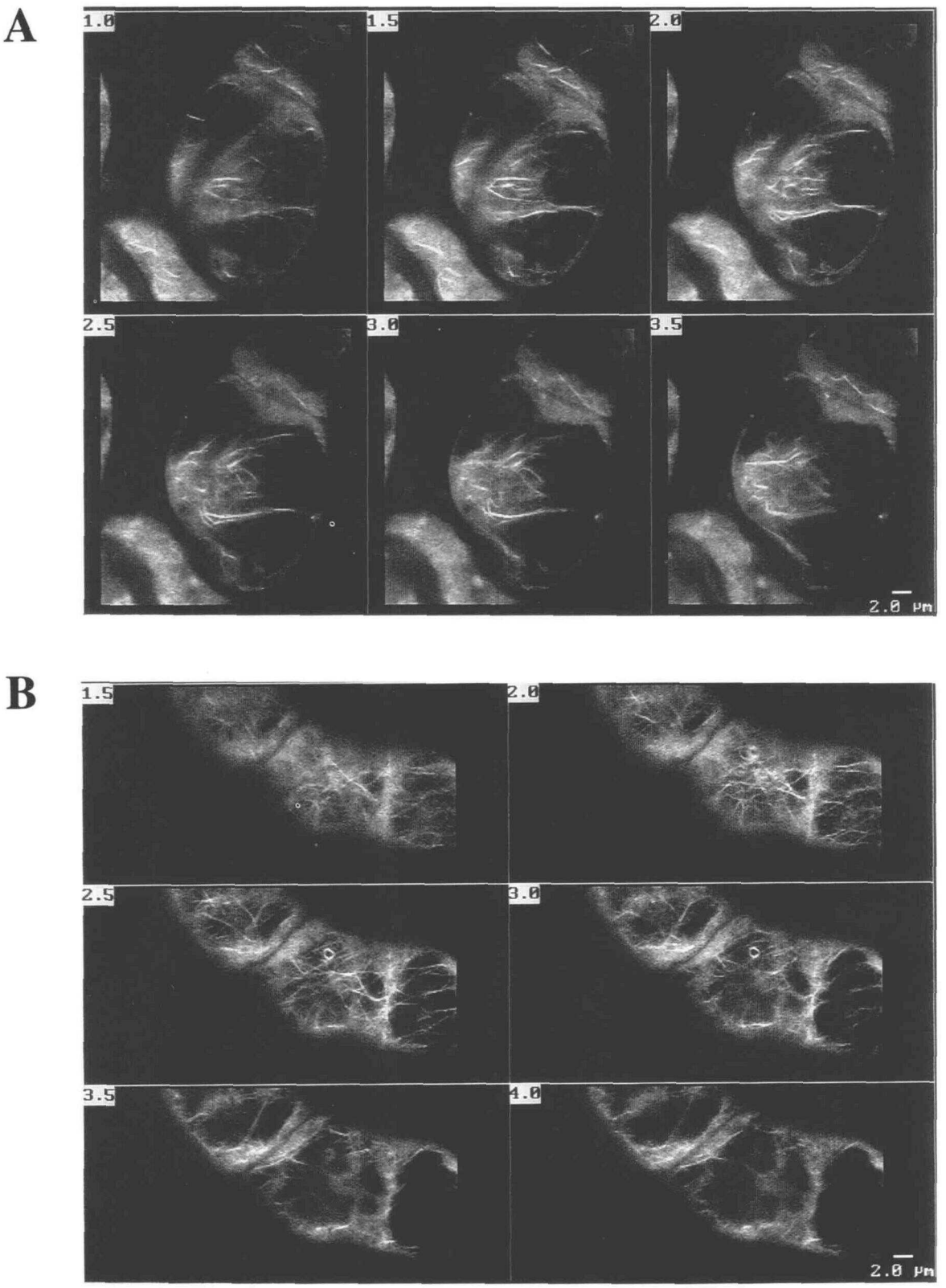

Figure 2. Confocal fluorescence imaging of the actin network in soybean root cells. Cells incubated with $AlCl_3$ (10 μM) are observed in A, and control cells are shown in B. The number in the top left corner of each image refers to the distance (μm) into the cell from the bottom closest to the objective. Bar represents 2 μm.

Table III. *Actin tension in the presence of EGTA, Ba^{2+}, and verapamil*

DT_{50}, Fifty percent point (10 successful displacements/20 attempts).

Effector	DT_{50}
	mW
Control (1B5C media)	15
EGTA (5 mM)	15
$BaCl_2$ (100 mM)	15
Verapamil	
(10 μM)	10
(100 μM)	10

availability of functional ATP–G-actin for actin polymerization (Fechheimer and Zigmond, 1993). As was also previously demonstrated for azide treatment (Pasternak and Elson, 1985; Pasternak et al., 1989; Grabski et al., 1994), an assembled F-actin network is required for aluminum to induce rigor. Pretreatment of soybean root cells with cytochalasin D prevented this effect (Table I).

Plant cells require dynamic actin- and tubulin-based networks for proliferation, differentiation, cell-plate formation, cell-wall biosynthesis, nuclear and vesicular migration, symbiosis, membrane recycling, and secretion (Gunning and Hardham, 1982; Lloyd, 1989). The pivotal role of the actin network in plant cell growth and viability suggests that aluminum-induced rigor may be a sufficient condition for cell death in proliferating cells within the root system of plants.

Received January 30, 1995; accepted April 27, 1995.

LITERATURE CITED

Alfrey AC, Mishell JM, Burks J (1972) Syndrome of dyspraxia and multifocal seize was associated with chronic hemodialysis. Trans Am Soc Artif Intern Organs **18:** 257–261

Driscoll CT Jr, Baker JP, Bisogni JJ Jr, Schofield CL (1980) Effect of aluminum speciation on fish in dilute acidified waters. Nature **284:** 161–164

Fechheimer M, Zigmond SH (1993) Focusing on unpolymerized actin. J Cell Biol **123:** 1–5

Foy CD, Chaney RL, White MS (1978) The physiology of metal toxicity in plants. Annu Rev Plant Physiol **29:** 511–566

Garruto RM, Yanagihara RT, Gajdusek DC (1990) Model of environmentally induced neurological disease: epidemiology of amyotrophic lateral sclerosis and parkinsonism-dementia in the western Pacific. Environ Geochem Health **12:** 137–151

Gassmann W, Schroeder JI (1994) Inward-rectifying K^+ channels in root hairs of wheat. Plant Physiol **105:** 1399–1408

Grabski S, Xie X-G, Holland JF, Schindler M (1994) Lipids trigger changes in the elasticity of the cytoskeleton in plant cells: a cell optical displacement assay for live cell measurements. J Cell Biol **126:** 713–726

Greger JL (1992) Dietary and other sources of aluminum intake. CIBA Found Symp **169:** 26–49

Gunning BES, Hardham AR (1982) Microtubules. Annu Rev Plant Physiol **33:** 651–698

Huang JW, Grunes DL, Kochian LV (1992) Aluminum effects on the kinetics of calcium uptake into cells of the wheat root apex. Quantitation of calcium fluxes using a calcium selective vibrating microelectrode. Planta **188:** 414–421

Kinraide TB, Arnold RC, Baligar VC (1985) A rapid assay for aluminum phytotoxicity at submicromolar concentrations. Physiol Plant **65:** 245–250

Koenig ML, Jope RS (1987) Aluminum inhibits the fast phase of voltage-dependent calcium influx into synoptosomes. J Neurochem **49:** 316–320

Konishi S, Miyamoto S (1983) Alleviation of aluminum stress and stimulation of tea pollen tube growth by fluorine. Plant Cell Physiol **24:** 857–862

LeVan H, Kuraishi S, Sakurai N (1994) Aluminum-induced rapid root inhibition and changes in cell-wall components of squash seedlings. Plant Physiol **106:** 971–976

Lloyd CW (1989) The plant cytoskeleton. Curr Opin Cell Biol **1:** 30–35

Macdonald TL, Humphreys WG, Martin RB (1987) Promotion of tubulin assembly by aluminum ion *in vitro*. Science **236:** 183–186

Macdonald TL, Martin RB (1988) Aluminum ion in biological systems. Trends Biochem Sci **13:** 15–19

Martin RB (1992) Aluminum speciation in biology. *In* Aluminum in Biology and Medicine. CIBA Foundation, John Wiley & Sons, New York, pp 5–25

McLachlan DR, Fraser PE, Dalton AJ (1992) Aluminum and the pathogenesis of Alzheimer's disease: a summary of evidence. *In* Aluminum in Biology and Medicine. CIBA Foundation, John Wiley & Sons, New York, pp 142–164

Pantoja O, Gelli A, Blumwald E (1992) Voltage-dependent calcium channels in plant vacuoles. Science **255:** 567–572

Pasternak C, Elson EL (1985) Lymphocyte mechanical response triggered cross-linking surface receptors. J Cell Biol **100:** 860–872

Pasternak C, Spudich JA, Elson EL (1989) Capping of surface receptors and concomitant cortical tension are generated by conventional myosin. Nature **341:** 549–551

Perl DP, Brody AR (1980) Alzheimer's disease: x-ray spectrometric evidence of aluminum accumulation in neurofibrillary tangle bearing neurons. Science **208:** 297–299

Ryan PR, Kinraide TB, Kochian LV (1994) Al^{3+}-Ca^{2+} interactions in aluminum rhizo toxicity. I. Inhibition of root growth is not caused by reduction of calcium uptake. Planta **192:** 98–103

Schenk RU, Bjorksten J, Yeager L (1989) Composition and consequences of aluminum in water, beverages, and other ingestibles. *In* TE Lewis, ed, Environmental Chemistry and Toxicology of Aluminum. Lewis Publishers, Chelsea, MI, pp 247–269

Schindler M (1995) The cell optical displacement assay (CODA): measurements of cytoskeletal tension in living plant cells with a laser optical trap. Methods Cell Biol **49:** 69–82

Short AIK, Winney RJ, Robson JS (1980) Reversible microcytic hypochromic anemia in dialysis patients due to aluminum intoxication. Proc Eur Dial Transplant Assoc **17:** 233

Siegel N, Haug A (1983) Aluminum interaction with calmodulin: evidence for altered structure and function from optical and enzymatic studies. Biochim Biophys Acta **744:** 36–45

Simpson W, Ellis HA, Kerr DNS, McElroy M, McNay RA, Peart KN (1976) Bone disease in long-term haemodialysis: the association of radiological with histological abnormalities. Br J Radiol **49:** 105–110

Thuleau P, Ward JM, Ranjeva R, Schroeder JI (1994) Voltage-dependent calcium-permeable channels in the plasma membrane of a higher plant cell. EMBO J **13:** 2970–2975

Viola RE, Morrison JF, Cleland WW (1980) Interaction of metal (III)-adenosine 5′-triphosphate complexes with yeast hexokinase. Biochemistry **19:** 3131–3137