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Effects of mechanical signaling on plant cell cytosolic calcium

(transgenic luminous plants/mechanoperception/recombinant aequorin/signal transduction)

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ABSTRACT **Mechanical signals are important influences on the development and morphology of higher plants. Using tobacco transformed with the Ca^{2+}-sensitive luminescent protein aequorin, we recently reported the effects of mechanical signals of touch and wind on the luminescence and thus intracellular calcium of young seedlings. When mesophyll protoplasts are isolated from these transgenic tobacco plants and mechanically stimulated by swirling them in solution, cytoplasmic Ca^{2+} increases immediately and transiently up to 10 μM, and these transients are unaffected by an excess of EGTA in the medium. The size of the transient effect is related to the strength of swirling. Epidermal strips isolated from transgenic tobacco leaves and containing only viable guard cells and trichomes also respond to the strength of swirling in solution and can increase their cytoplasmic Ca^{2+} transiently up to 10 μM. Finally, the moss *Physcomitrella patens* containing recombinant aequorin exhibits transient increases in cytoplasmic Ca^{2+} up to 5 μM when swirled in solution. This effect is strongly inhibited by ruthenium red. Our data indicate that the effect of mechanical stimulation can be found in a number of different cell types and in a lower plant as well as tobacco and suggest that mechanoperception and the resulting increase in cytoplasmic Ca^{2+} may be widespread.**

It has been known for many centuries that plants are sensitive to mechanical stimulation, although it has been commonly assumed that mechanical responses are limited to such obvious examples as *Mimosa* or *Dionea* (1). However, compendia prepared by Darwin himself and others in the last century indicated the wide range of different plant species which respond to touch as a mechanical signal via sensitive stems, petioles, flower peduncles, stamens, and even roots (2–4). The majority of responses to mechanical signals result in reduction of growth rates and thickening of cell walls (usually by lignification), thus stiffening tissues. Mechanically stimulated crop plants have much lower yields than unstimulated plants, probably resulting from the diversion of carbohydrates into the lignified walls rather than into vertical growth, fruit, or seed production. However, there are also many reasons to associate mechanoperception and response with fundamental mechanisms of morphogenesis (1). Mechanical stimulation may also be important in resistance to fungal invasion. Many responses to invading fungi involve lignification, and the infection peg may be involved in pressure signaling on responding cells. The mechanisms of mechanical perception and transduction are not understood, but current research indicates that perception of mechanical stimuli occurs via integrins and that transduction of the mechanical signal involves changes in the cytoskeleton (1).

Our own interest in understanding the transduction of mechanoperception arose from studies on cytoplasmic Ca^{2+}. We developed a method for measuring cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$), utilizing transformation of tobacco plants with the cDNA for the Ca^{2+}-sensitive protein aequorin (5, 6). Thus, luminous plants were produced whose luminosity directly reported $[Ca^{2+}]_i$. One of the earliest signals found to immediately and transiently increase $[Ca^{2+}]_i$ was the mechanical signal of touch (5). Later studies showed that wind stimulation of transgenic seedlings immediately and transiently increased $[Ca^{2+}]_i$ in a dose-dependent fashion (6). Both touch and wind probably signal plants by causing tissue movement resulting in differential changes in tension and compression across tissues (1). Which cells actually respond to touch or to wind is not known, although luminescence imaging studies (7) have shown that some cotyledon cells can be very sensitive to even slight touch stimulation.

One disadvantage with using whole tobacco seedlings for such work is the difficulty of defining precisely which cells actually respond to mechanical stimulation. If different tissues respond uniquely to mechanical stimuli, the specificity of the response may be lost in the inevitable whole plant averaging. A further problem is our inability to easily calibrate the size of the $[Ca^{2+}]_i$ change which accompanies mechanical stimulation. The majority of luminescence from these luminous seedlings probably emerges from the epidermis, with reduced contributions from subepidermal cells. Both of these difficulties have driven us to seek simpler alternatives in which the basis of mechanical signaling can be more easily dissected. It is the purpose of this paper to describe several such examples.

MATERIALS AND METHODS

Plant and Tissue Material, Preparation, and Transformation. The previously transformed *Nicotiana plumbaginifolia* (pMAQ2) containing the cDNA for apoaequorin under control of the cauliflower mosaic virus 35S promoter was used throughout this study (5, 6). Protoplasts were isolated by standard enzymatic methods (8) from the leaves of 8-week-old plants grown in white light (100 μmol·dm^{-2}·s^{-1}) for a 16-hr day at 24°C. The isolation medium contained 400 mM sucrose, 1% cellulase, 0.2% macerozyme, and 0.1 mM $CaCl_2$ (pH 5.6) and protoplasts were washed three times after overnight incubation in 400 mM sucrose/10 mM Mes (pH 5.7), yielding a final

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Abbreviation: $[Ca^{2+}]_i$, cytoplasmic Ca^{2+} concentration.

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solution with about 10^6 viable protoplasts per ml. Viability was assessed by fluorescein diacetate staining (9).

Epidermal peels were made from the 8-week-old transformed *N. plumbaginifolia* leaves described above and incubated in 50 mM KCl/20 mM NaH_2PO_4 buffer (pH 5, adjusted with phosphoric acid) with aeration for several hours in the darkness. Viability tests using the vital stain fluorescein diacetate and the mortal stain propidium iodide indicated that this treatment killed all epidermal cells but left guard cells and trichomes intact and, in the case of guard cells, with the ability to open and close.

The moss *Physcomitrella patens* was maintained on simple salts medium as described (10). For purposes of transformation, protoplasts were isolated by a 1-hr treatment with driselase (11). Protoplasts were transformed with the construct pMAQ2 used for transformation of *N. plumbaginifolia* (5, 6) by incubation in 5% PEG and heat shock (12, 13). After limited regeneration, stable transformants were selected for resistance to kanamycin (100 μg/ml) and protein extracts were subjected to Western blotting with anti-apoaequorin (5) to demonstrate the presence of apoaequorin in the transformed lines.

Aequorin Reconstitution and Luminescence Measurements. Tissues were incubated with 2 μM coelenterazine for 2–4 hr at room temperature unless stated otherwise. After washing, luminescence measurements were made with a digital chemiluminometer equipped with an EMI photomultiplier, type 9924A at 1 kV, and a discriminator (6) with recording facilities. With epidermal strips the amount of reconstituted aequorin can vary substantially between similar-sized strips. However, strips providing $<10^5$ photons of detected aequorin were discarded. Sample sizes of other tissues were chosen so that luminescence of total aequorin was $>10^5$ photons in 10 s.

Calibration of $[Ca^{2+}]_i$ Transients After Stimulation. At the end of each experimental treatment of the samples of protoplasts, epidermal strips, or *Physcomitrella*, the total remaining aequorin was discharged and estimated by mixing with an excess of a solution of 10% ethanol/900 mM $CaCl_2$ in the luminometer. This was the only solution found which would discharge within seconds the cellular aequorin in epidermal strips and *Physcomitrella*; however, protoplasts were discharged with 100 mM $CaCl_2$/2% (vol/vol) Nonidet P-40. The size of the experimentally induced $[Ca^{2+}]_i$ transient could thus be calculated by using the ratio of consumed aequorin to total aequorin as described by Cobbold and Rink (14).

RESULTS

Effect of Wind on Transgenic Seedling Luminescence. In a previous publication (6) we reported that wind stimulation immediately and transiently increased $[Ca^{2+}]_i$ as monitored by luminescence. In those experiments we stimulated the transformed seedlings by subjecting them to immediate blasts of air from a syringe. The size of the stimulus was varied by simply increasing the volume of air used for stimulation. In the present study we stimulated seedlings by using a constant volume of air (50 ml) released over 2, 3, 4, or 5 s (Fig. 1). Again there was a threshold rate of stimulation (rate must be >5 s) and thereafter a relationship between the speed at which the air was released and the size of the luminescent signal. The inability to expel 50 ml of air in 1 s prevented the measurement of this stimulation.

Effect of Mechanical Stimulation on Mesophyll Protoplast Luminescence. To test the possible sensitivity of individual cells to mechanical stimulation, we isolated mesophyll protoplasts from transgenic leaves and reconstituted the aequorin by incubation with coelenterazine for 3 hr. Protoplast samples (0.1 ml) were placed in the luminometer and stimulated by adding 0.5 ml of isotonic incubation medium in 1, 2, 3, 4, or 5 s via a syringe. The resultant effects on luminescence (Fig. 2*a*) show large $[Ca^{2+}]_i$ transients (up to 10 μM) with the 1-s addition, dropping away very rapidly with the slower addition of the sucrose solution.

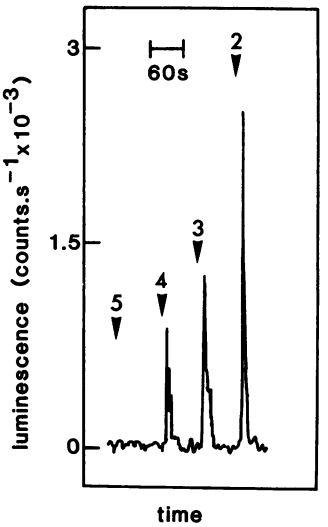

FIG. 1. Effect of wind stimuli of increasing force on cytosolic Ca^{2+}-responsive luminescence of *Nicotiana* seedlings. A trace from a chart recorder connected to a chemiluminometer shows the changes in luminescence of a seedling exposed to 50 ml air from a syringe expelled in 5, 4, 3, or 2 s. Air was applied at the times indicated by the arrowheads.

After the swirling treatment the protoplasts were lysed as described and the $[Ca^{2+}]_i$ transient was calibrated. All experiments were repeated five times (with five replicates in each case). The traces shown have been chosen to best represent the average result.

We have made estimates of the actual force involved in the addition of solution as described before for wind (6). The 1-s addition applied a force of ≈6 N to the protoplast sample above the actual weight of solution added and led to immediate mixing as observed with colored solutions; the 2-s addition involved a force of 1.3 N above the weight added and mixing

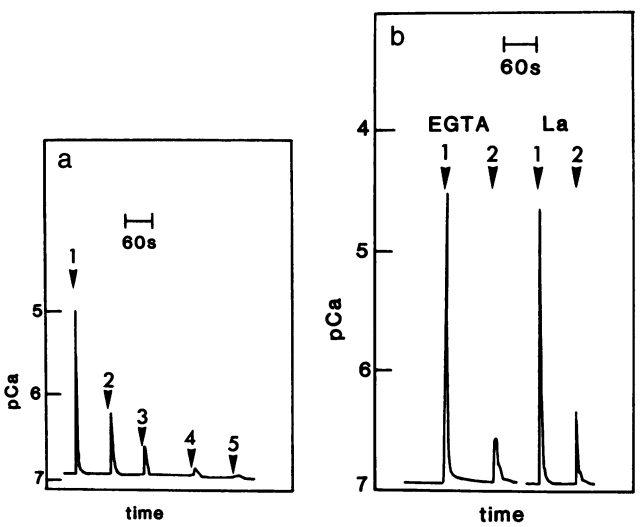

FIG. 2. Influence of mechanical stimulation on tobacco protoplast cytosolic Ca^{2+}-responsive luminescence. Traces from a chart recorder connected to a chemiluminometer show the changes in protoplast luminescence. (*a*) Approximately 10^5 protoplasts suspended in 0.15 ml of medium mechanically stimulated by injection at arrow of 0.5 ml of isotonic medium over 1, 2, 3, 4, or 5 s from a syringe. (*b*) Protoplasts preincubated in 1 mM EGTA or 1 mM La^{3+} for 0.5 hr before mechanical stimulation with 0.5 ml of isotonic medium injected over a period of 1 or 2 s.

continued for about 0.5 s after the 2-s addition. The 3- to 5-s additions gave no detectable force above the weight of solution added and the additional mixing period was 1, 2, and 2.5 s, respectively, after the addition of the solution.

Since very vigorous addition of solution could cause protoplast damage, leading to discharge of aequorin from lysed protoplasts, we estimated viable protoplast numbers (using fluorescein diacetate and a hemocytometer) before and after addition of the isotonic incubation medium, but correcting for volume changes. Before mixing the protoplasts were 105 ± 3.5 cells per 0.1 μl; after 1-s addition of solution, 97.6 ± 6.6 cells per 0.1 μl; and after 5-s addition, 96.5 ± 8.2 cells per 0.1 μl. Thus the protoplast numbers are not significantly different from one another as a result of vigorous or slow mixing and indicate that the $[Ca^{2+}]_i$ transient is not the result of protoplast lysis.

Further evidence that the luminescence spike does not result from protoplast lysis can be deduced from Fig. 2*b*. Protoplasts were preincubated for 30 min in 1 mM EGTA in the absence of Ca^{2+} and then stimulated by isotonic solution addition; only the 1- and 2-s additions are shown. In this case the $[Ca^{2+}]_i$ transient is consistently higher than in medium without EGTA. Aequorin from lysed protoplasts would not have been discharged during this treatment. Experiments have also been performed in which the protoplasts were preincubated with equal amounts of Ca^{2+} and EGTA (data not shown), and the transient up to 10 μM was still observed. We have also carried out an extensive set of measurements using La^{3+} and Gd^{3+} in the incubation medium at a range of concentrations and ruthenium red up to 100 μM (data not shown). An example of 1 mM La^{3+} in the presence of 1 mM Ca^{2+} is shown in Fig. 2*b*. No inhibition of the mechanical stimulation of protoplast $[Ca^{2+}]_i$ by these inhibitors and Gd^{3+} was observed.

Mechanical Stimulation of Epidermal Strips. Epidermal strips were isolated from transgenic tobacco leaves and incubated with coelenterazine for 4 hr, resulting in maximum formation of aequorin. Tests with fluorescein diacetate indicated that under the incubation conditions used (pH 5), only guard cells and trichomes were viable. The guard cells open under these conditions and can be closed by addition of abscisic acid (data not shown). Fig. 3*a* shows the effect of adding incubation medium at different rates (1, 2, 3, and 5 s) to the epidermal strip on $[Ca^{2+}]_i$. After each incubation the total aequorin was discharged and the $[Ca^{2+}]_i$ transient was estimated. Visible observation indicated that the strips remained intact with these mixing protocols. Each experiment has been replicated at least 5–10 times and the traces selected best represent the average.

Even with slower additions of medium to these strips, substantial increases in $[Ca^{2+}]_i$ were detected, although there was a clear drop in the size of the transient as medium addition slowed from 1 to 3 s. However, even after the slow 5-s addition, $[Ca^{2+}]_i$ transients up to 1 μM were still detected. Again visual observation showed these strips to have very little tensile strength since the epidermal cells were dead and had lost turgor. The epidermal strip shape was transiently changed as solution is added to it even with the slowest addition (5 s), and this could have led to tension and compression changes on the viable guard cells and trichomes.

As with the protoplasts we have used a number of inhibitors to investigate these $[Ca^{2+}]_i$ transients induced by mechanical stimulation. Fig. 3*b* shows the effect of preincubating strips in 1 mM EGTA, 1 mM La^{3+}, and 50 μM ruthenium red. None of these pretreatments had detectable effects on the transient induced by a 1-s solution addition.

Effect of Mechanical Stimulation on *Physcomitrella* $[Ca^{2+}]_i$. *Physcomitrella patens* has been transformed with the plasmid used for transformation of tobacco (A.J.R., unpublished work). Petri dishes contained up to 1 g of *Physcomitrella* protonema, grown on a simple mineral salts medium with only 1

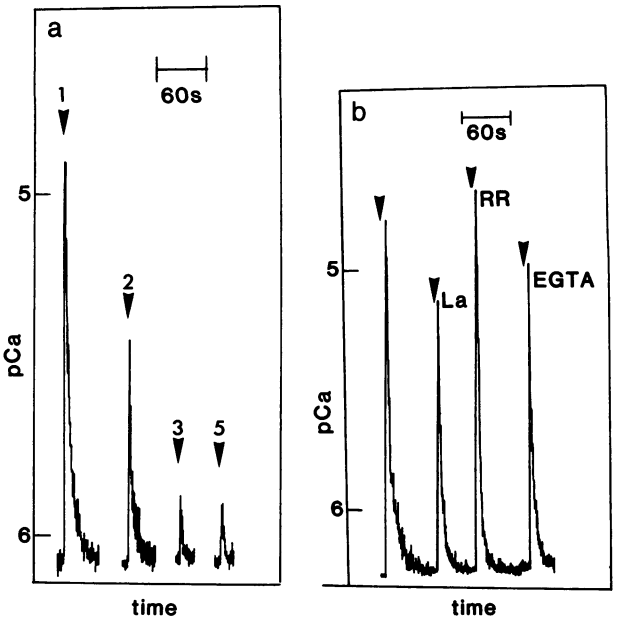

FIG. 3. Influence of mechanical stimulation on Ca^{2+}-responsive luminescence of tobacco leaf epidermal strip. Traces from a chart recorder connected to a luminometer show changes in epidermal strip luminescence. (*a*) Epidermal strips mechanically stimulated by injection (arrowheads) of 0.5 ml of 50 mM KCl/20 mM NaH_2PO_4 (pH 5.0) over 1, 2, 3, or 5 s. (*b*) Epidermal strips preincubated for 0.5 hr in the absence of inhibitors or the in presence of 1 mM La^{3+}, 50 μM ruthenium red (RR), or 1 mM EGTA before mechanical stimulation by injection of 0.5 ml of medium over 1 s.

μM Ca^{2+} and consisting of a filamentous mat. These were scraped into coelenterazine and incubated for 4 hr to reconstitute aequorin. Samples of 10–20 mg were placed in tubes in the luminometer and stimulated by application of 0.5 ml of water through a syringe for 1–5 s. All measurements were replicated five times and several separate experiments were conducted for each treatment.

Fig. 4 shows data for only 1 and 3 s. The pattern is very similar to the situation with protoplasts, with a rapid drop in luminescence between the 1- and 2-sec stimulations. The transients induced in *Physcomitrella* were lower than those observed with the other two tissues but were still up to ≈5 μM. Again, visual observation showed that the 1-s treatment led to extensive movement of tissue filaments with respect to each other, but at 3 s movement was difficult to observe and the network of filaments seemed largely stabilized against movement which would result in changes in tension and compression.

The effects of inhibitors and addition of Ca^{2+} on the 1-s-induced luminescence were also tested (Fig. 4). Inhibitors (or Ca^{2+}) were added during the incubation with coelenterazine. Results for $CaCl_2$ (25 μM), EGTA (1 mM), $LaCl_3$ (10 mM), and ruthenium red (100 μM) are shown. EGTA and La^{3+} caused some inhibition of the response. We used 10 mM La^{3+} because little effect was obtained at 1 mM. Surprisingly, prior incubation in 25 μM Ca^{2+} also reduced the size of the transient, although the reductions were not large. Pretreatment with ruthenium red led to total abolition of the response.

DISCUSSION

This paper describes the effects of apparent mechanical stimulation on protoplasts and epidermal strips from transgenic tobacco and from a moss *Physcomitrella*. In each case the tissues or cells respond by an immediate and transient increase in $[Ca^{2+}]_i$ and the size of the transient is related in part to the

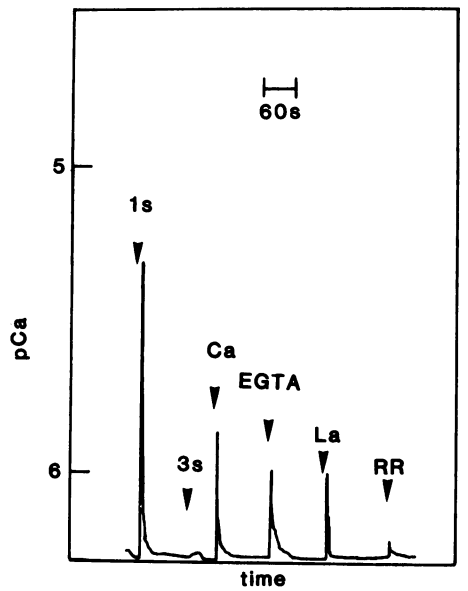

FIG. 4. Effect of mechanical stimulation on *Physcomitrella* cytosolic Ca^{2+}-responsive luminescence. Trace is from a chart recorder connected to a chemiluminometer. *Physcomitrella* was mechanically stimulated by injection of 0.5 ml of water (arrowheads) over 1 or 3 s. *Physcomitrella* was preincubated for 0.5 hr with 25 μM Ca^{2+}, 1 mM EGTA, 10 mM La^{3+}, or 0.1 mM ruthenium red (RR) before being mechanically stimulated by injection of 0.5 ml of water over 1 s.

energy or force with which the stimulus is administered. However, what direct mechanical effect the stimulus is having in each of these tissues or plants is not clear.

In our previous paper (6) we were able to show a relationship between $[Ca^{2+}]_i$ response and seedling movement caused by wind stimulation. Wind stimulation caused the seedling to pivot around the root hypocotyl junction. The longer the plant was in motion the greater the observed luminescence. However, we do not know whether increasing the strength of the stimulus merely recruited a greater number of cells into a $[Ca^{2+}]_i$ response or whether it reflected a higher level of $[Ca^{2+}]_i$ in the same number of responding cells. Furthermore, we do not know which cells in the seedling luminesced in response to wind. Our present results suggest that it was tissue bending which induced the luminescence. Pfeffer (4) concluded that mechanical stimulation relied upon a localized shearing or differential deformation of a tissue—i.e., tension in some cells and compression in others.

Localized deformation is more difficult to visualize in the case of protoplasts. The effects of immediate mixing might propel protoplasts to impact on the walls of the tube, resulting in temporary deformation. At the protoplast densities used, hitting each other is less likely. However, if protoplasts do impact this does not lead to protoplast rupture. Deformation of the external membrane might initiate stretch channel activity, which has been suggested to transduce mechanical stimuli (15). However, we found that the effect of mixing on $[Ca^{2+}]_i$ was not inhibited by La^{3+} or Gd^{3+} or EGTA, all of which should inhibit the entry of external Ca^{2+} into the protoplast. We cannot exclude a possible internal deformation of, for example, the vacuole leading to increased stretch channel activity. There should be a mechanism in plant cells for sensing turgor pressure and this might rely on sensing cell shape. Deformation caused by impact might then activate this mechanism.

An alternative is that rapid protoplast movement caused by mixing initiates shearing forces between the outer membrane and the solution. *Fucus* zygotes respond to the rate of fluid flow and can use this signal to specify the direction of the polar axis (16). There is much evidence to relate the formation of the axis to $[Ca^{2+}]_i$ changes. A further alternative is that rapid mixing of the solution might lead to turbulent flow and temporary pressure changes which transmit directly to the protoplast, in a manner perhaps akin to sympathetic vibration. Many animals possess cells which respond to fluid pressure changes (e.g., in the ear), but here again it is thought that plasma membrane stretch channels are involved (17).

There are many unicellular organisms which respond to the simple mechanical stimulation of being shaken (18). Many of these are found in the surface waters of phosphorescent seas and respond to this minor mechanical stimulation by luminescing. It is thought that the luminescent signal is induced by a change in cytoplasmic pH (19). Since plant vacuoles have been reported to contain a Ca^{2+}/H^+ antiport (20), changes in cytosolic pH could explain the transient changes in $[Ca^{2+}]_i$ observed here. Cultured cells of *Arabidopsis* transformed with aequorin have also been observed to glow when shaken (Janet Braam; personal communication). Since these cells possess a rigid cell wall, deformation and localized shearing between the wall and the plasma membrane may explain the results in this case.

We were unable to obtain inhibition of the protoplast $[Ca^{2+}]_i$ transient with ruthenium red. Since we did obtain inhibition of wind-induced $[Ca^{2+}]_i$ with ruthenium red (6), this suggests a difference between the two processes. The inhibitory site of action of ruthenium red is not understood. Marshall *et al.* (21) reported that ruthenium red could inhibit Ca^{2+} uptake by plasma membrane vesicles, but the inhibitor is generally thought to have an intracellular mode of action (22). Since the inhibitor can be rapidly taken up into plant cells, both possibilities are open.

Epidermal strips present a slightly different perspective. In response to an increasing energy input into the solution a higher $[Ca^{2+}]_i$ transient is induced, but this is less dramatic than for the protoplast (compare Figs. 2 and 3). Even at very slow rates of addition of the medium to the epidermal strip a transient up to 1 μM can be detected. The epidermal strip has little rigidity because the epidermal cells have lost turgor. Consequently, transient changes in shape easily occur even with slight fluid movement. It is possible in this case that the Pfeffer requirement (4) of localized shearing and deformation could apply.

The epidermal strips contain only viable guard cells and trichomes. It is perhaps easier to visualize the trichomes as the cells that are sensitive to fluid movement and even to slight movement. Epidermal hairs have been considered to have a largely passive function in the physiology of the shoot, being mainly concerned with controlling humidity around the leaf or inhibiting aphid movement. If it can be shown that the hairs do respond mechanically to movement by increasing $[Ca^{2+}]_i$, then this might help us to reappraise their significance to leaf and stem physiology. Transmission of signals might follow as a consequence. On the other hand, if guard cells are responsive to mechanical deformation or fluid flow, this has important consequences for stomatal physiology.

Use of a range of inhibitors on the epidermal strip suggests that it is mobilization of intracellular Ca^{2+} which is responsible for the transient luminescence induced by movement (Fig. 3). Again we have no indication of the mechanism involved, although in this case the cells which luminesce have an intact cell wall. Our data suggest that stretch channels, or other Ca^{2+} channels, in the plasma membrane are not responsible for the transduction of the mechanical stimulus.

The results with transformed *Physcomitrella* are different again. Mechanical stimulation induced $[Ca^{2+}]_i$ transients but only with the most vigorous stimulus. The tissue is a filamentous mat. The mat itself forms a mesh—a network of filaments—and the filaments are composed of strings of single cells joined together. The network character of the mat may act

to dampen filament movement, except when the stimulus is vigorous and the strings can flex. Flexing itself will produce the localized shearing and deformation which Pfeffer (4) described as necessary for mechanical stimulation. However, the effects of inhibitors point to a different mechanism for induction of the $[Ca^{2+}]_i$ transient compared with protoplasts and epidermal strips. EGTA and La^{3+} do cause some reduction in the transient, although a high concentration, 10 mM, is necessary in the case of La^{3+}. External sources of Ca^{2+} may therefore contribute at least in part to the resulting $[Ca^{2+}]_i$ transient. However, the only source of Ca^{2+} available to the plants we used was in the cell wall. *Physcomitrella* was grown on a medium containing only 1 μM Ca^{2+}, with no apparent ill effects. In addition, when the cultures were stimulated this was carried out with distilled water. With culture preincubation in 25 μM Ca^{2+} there was in fact slight inhibition of the $[Ca^{2+}]_i$ transient, an unexpected result if the external medium was contributing to the $[Ca^{2+}]_i$ transient. An alternative explanation of this latter result is that the Ca^{2+} acts to stiffen the cell wall and thus reduce the extent of flexing of the filaments in a moving solution. Ruthenium red, however, completely blocked the mechanically induced $[Ca^{2+}]_i$ transients. The inhibitor sensitivity of these transients, slight to La^{3+} but strongly inhibited by ruthenium red, is similar to those we detected with tobacco seedlings. One interpretation is that a major contribution of the transduction of the mechanical stimulus is occurring inside the cell. Transformation of *Physcomitrella* with targeted aequorin might help resolve the source of Ca^{2+} involved here.

These three examples, together with those of wind and touch stimulation examined previously (5, 6), do not lend much support to the notion of a plasma membrane-localized transduction of mechanical signals. Much exciting research has indicated that integrins, proteins which link the cell membrane to the cytoskeleton, are primary receptors of mechanical stimulation in animal cells (23). One of the earliest events following integrin signaling is an increase in $[Ca^{2+}]_i$, and it is supposed that this involves, rather directly, enhanced movement of Ca^{2+} across the plasma membrane. The mechanical sensing devices used by plant cells are certainly not in any way understood, but there have been suggestions that plant equivalents of integrins are involved. Indeed Pickard and Ding (15) have suggested that integrins might directly influence the activity of plasma membrane stretch channels in plant cells. While our data say nothing at all about the location of plant cell mechanoperception, an initial sensing at the plasma membrane seems most likely. However, a primary component of the induced $[Ca^{2+}]_i$ transient seems to originate within the cell. A simple explanation might involve inositol trisphosphate synthesis and release from the plasma membrane. In this case an effect of mechanical signaling on plasma membrane phospholipase C activity should be investigated.

The role of mobilization of $[Ca^{2+}]_i$ as a result of mechanical signals seems likely to be the disruption of a previous cytoskeleton, allowing a new structure to be rebuilt. This should be seen as a necessary precondition of adaptation to the mechanical response. In turn this could lead to the induction of gene expression of necessary enzymes or the induction of touch genes such as calmodulin (24). Since Ca^{2+} is not a highly mobile ion in the cytoplasm, the precise spatial location of $[Ca^{2+}]_i$ increases becomes an important element in determining the specificity of a response. Determining the subcellular origin of the Ca^{2+} changes described here becomes the next crucial step if we are to understand the transduction of mechanical stimuli.

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