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# Mechanical signalling, calcium and plant form

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**Abstract**

Calcium is a dynamic signalling molecule which acts to transduce numerous signals in plant tissues. The basis of calcium signalling is outlined and the necessity for measuring and imaging of calcium indicated. Using plants genetically transformed with a cDNA for the calcium-sensitive luminescent protein, aequorin, we have shown touch and wind signals to immediately increase cytosol calcium. Touch and wind signal plant cells mechanically, through tension and compression of appropiate cells. Many plant tissues and cells are very sensitive to mechanical stimulation and the obvious examples of climbing plants, insectivorous species as well as other less well-known examples are described. Touch sensing in these plants may be a simple evolutionary modification of sensitive mechanosensing system present in every plant. The possibility that gravitropism may be a specific adaptation of touch sensing is discussed. There is a growing appreciation that plant form may have a mechanical basis. A simple mechanical mechanism specifying spherical, cylindrical and flat-bladed structures is suggested. The limited morphological variety of plant tissues may also reflect mechanical specification. The article concludes with a discussion of the mechanisms of mechanical sensing, identifying integrin-like molecules as one important component, and considers the specific role of calcium.

## Calcium acts as a universal signalling molecule

Calcium is the most dynamic of known signalling molecules. No other signalling molecule receives such attention or is the subject of such intense interest. The use of technologies which image the distribution of calcium in single cells have uncovered an enormous variety of signalling mechanisms. These discoveries have illustrated the diverse way in which calcium acts to fundamentally regulate metabolism and development and must stand as one of the prime achievements of biological research in the past decade.

Figure 1 is a simple cartoon which outlines events in calcium signalling. Such diagrams are not intended to be realistic descriptions of calcium signalling but do describe calcium signalling at one level of understanding. Calcium enters the cytosol through specific calcium channels – protein pores whose existence in plant cells is now clearly established by patch clamp studies (see references and discussion in [18, 55]). The resting level of the $Ca^{2+}$ in the cytoplasm is about 100 nM and is maintained at this very low level by plasma membrane and other $Ca^{2+}$ ATPases [16]. Upon signalling, intracellular calcium, $[Ca^{2+}]_i$, in the cytosol is increased and combines with calmodulin, the primary plant calcium

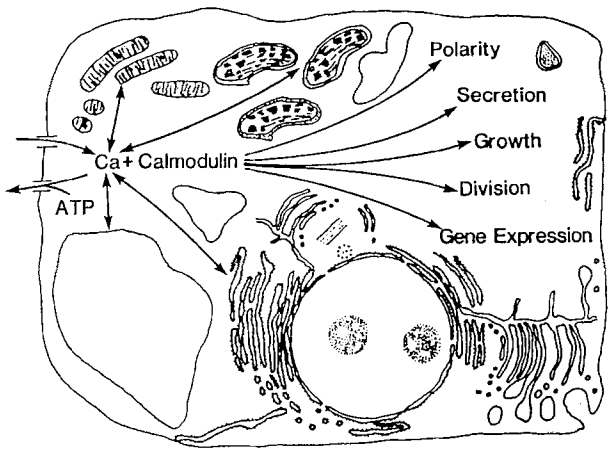


*Fig. 1.* A cartoon summarising the basic elements of calcium signalling. The cartoon illustrates the entry of extracellular calcium through plasma membrane bound channels in to the cytoplasm, expulsion via plasma membrane-located $Ca^{2+}$ ATPases and interrelations with calcium in other organelles. Combination with calmodulin the primary calcium receptor influences numerous cellular responses.

receptor, or other calcium-binding proteins. Subsequent cellular processes are then initiated usually via activation of appropiate protein kinases.

The cytosolic $Ca^{2+}$ pool is in communication with storage pools in the vacuole and the rough endoplasmic reticulum (ER) which may contain up to 10–1000 mM $Ca^{2+}$. Both the vacuole and the rough ER membranes contain $Ca^{2+}$ channels and ATPases and $Ca^{2+}$ mobilisation may also be regulated via $Ca^{2+}/H^{+}$ exchange.

The situation with mitochondria, chloroplasts and nuclei is less clear. Originally these organelles were regarded as merely helping to regulate cytosolic $[Ca^{2+}]_i$ and to act as $Ca^{2+}$ stores. Increasingly, the perception is that intraorganelle $[Ca^{2+}]_i$ may itself manipulate oxidative respiration, photosynthetic $CO_2$ fixation and, most notably, gene expression [2, 44, 45, 61]. These three organelles have all been shown to contain calcium-binding proteins and calmodulin. This would suggest that $[Ca^{2+}]_i$ acts as a co-ordinating molecule able to mobilise both cytoplasmic and organelle activities after signalling. Of these three organelles there is currently most interest in the nucleus. This organelle can maintain a free $Ca^{2+}$ concentration different to the cytosol, it can signal directly through its own calmodulin and possesses the enzymology to hydrolyse phosphatidylinositol 4,5-bisphosphate ($PIP_2$), synthesising the calcium mobilising agent, inositol 1,4,5-trisphosphate ($IP_3$) [2, 61].

Although we are still unsure of many of the details of calcium signalling we are sure of its importance in the transduction of a variety of signals in plant cells. The Molecular Signalling Group at Edinburgh has employed fluorescence ratio imaging and photometry of $[Ca^{2+}]_i$ coupled with UV photolysis of caged $Ca^{2+}$ and caged $IP_3$ to mimic observed $[Ca^{2+}]_i$ transients. In one case caged abscisic acid has also been used [1]. These results establish clearly an involvement of $[Ca^{2+}]_i$ in stomatal aperture control, red light transduction through phytochrome, pollen/stigma incompatibility and the direction of pollen tube growth [1, 17, 19, 43, 60]. Other data [46, 50, 56] clearly establish the presence of a calcium gradient in pollen tubes which is required for the maintenance of growth. We have described numerous other signals which modify $[Ca^{2+}]_i$ but these observations do not yet have the necessary confirmation of significance using caged calcium release [36–38]. Although such methods establish an involvement of $[Ca^{2+}]_i$ in transduction they do not debar the involvement of other signalling processes. Signal transduction pathways are complicated networks with numerous parallel and interlinked events; the proportion of flux through each of the main pathways shifts as circumstances change [1].

Much early scepticism of the significance of $[Ca^{2+}]_i$ signalling concerned the large variety of signals $[Ca^{2+}]_i$ was believed to transduce. There is not much variety available in the kinetics of concentration; it can increase, decrease or stay the same. Some additional variety can be introduced through signalling-induced relocation of calmodulin or release of calmodulin from bound forms by phosphorylation [42]. Furthermore, a variety of calmodulins with different sequences and different cell locations increase possible complexity in transduction [5]. A novel signalling mechanism, the induction of waves of $[Ca^{2+}]_i$

and frequency modulation of waves by different signals, represents a further rich source of signalling and transduction information [31]. Further complexity follows from the spatial constraints exerted on the movement of $[Ca^{2+}]_i$ in the cytoplasm. Estimates of the $[Ca^{2+}]_i$ diffusion constant suggest it to be 100-fold lower than in free solution [3]. This diffusional constraint is believed to result from the presence of numerous calcium-binding proteins attached to the cytoskeleton or located on membrane structures. As a consequence, transient signalling, which is localised to regions of the plasma membrane (for example by receptor clustering), initiates an equally localised response to regions of adjacent cytoplasm. Although we in the Molecular Signalling Group have made many hundreds of observations of the distribution of $[Ca^{2+}]_i$ in signalled plant cells, we have yet to observe cells in which $[Ca^{2+}]_i$ is uniformly distributed, except in the resting state. Even ionophores do not produce even distributions of $[Ca^{2+}]_i$. Finally, cell age determines the cellular metabolic apparatus capable of responding to a change in $[Ca^{2+}]_i$, increasing the variety of specific signalling mechanisms available.

Figure 1 emphasises the necessity of measurement and the imaging of $[Ca^{2+}]_i$ in order to understand calcium signalling. That emphasis may be seen to be misplaced as further detailed knowledge emerges, but it provides a route towards an improved understanding.

## Touch and wind signals initiate immediate increases in $[Ca^{2+}]_i$

The Edinburgh Molecular Signalling Group has developed a novel method for measuring and imaging $[Ca^{2+}]_i$. Aequorin is a calcium-sensitive luminescent protein from the jellyfish *Aequorea victoria*. In the yellyfish high levels of aequorin are found in specific cells. When attacked by a predator, $[Ca^{2+}]_i$ rapidly elevates in these cells causing the jelly fish to luminesce, thus inhibiting predation.

Aequorin is a 21 kDa protein composed of an apoprotein, apoaequorin, and an imidazolopyrazine luminophore, coelenterazine. On interaction with $Ca^{2+}$, coelenterazine is oxidized to coelenteramide and luminescent light is emitted. Calibration of emitted light against free $Ca^{2+}$ is relatively straightforward in free solution and in single cells [38]. There are numerous types of coelenterazine available which form aequorins with very different properties (for references and use, see [38]). Calibration of $[Ca^{2+}]_i$ transients can be conducted using e-coelenterazine which forms an aequorin with a bimodal spectrum. Luminescence ratio measurements at two different wavelengths can be linearly related to $Ca^{2+}$ concentration freeing $[Ca^{2+}]_i$ measurements from variations in aequorin concentration.

We have genetically transformed *Nicotiania plumbaginifolia* to express apoaequorin and showed that incubation of the transformed seedlings in coelenterazine led to reconstitution of actively reporting aequorin [36]. Since aequorin is a soluble protein we have thus constituted luminous plants whose luminosity directly reports cytosolic $[Ca^{2+}]_i$. The aequorin was expressed in all three primary regions of the seedling, the cotyledons, stem and root. Targeting of aequorin to different cell compartments or different cell types, different tissues or different developmental ages provides a $[Ca^{2+}]_i$ measuring method of extraordinary range and power.

These transformed seedlings were found to be touch-sensitive [36]. By inserting a fine wire through the luminometer port and arranging a transformed seedling so that a kink in the wire touched it on one rotation, we were able to observe (transient) bursts of luminescent light each time the seedling was touched (Fig. 2). Touch therefore immediately increased $[Ca^{2+}]_i$.

We were able to observe that the seedlings we touched by this method showed slight movement. Was the increase in $[Ca^{2+}]_i$ the result of movement or did it result from touch damage of hairs and epidermal cells? To clarify this situation we sought to induce movement by means which did not involve touch. Seedlings were fixed in the luminometer by their roots and subjected to small blasts of air from a syringe mimicking a wind stimulation [37]. These stimuli caused the plant

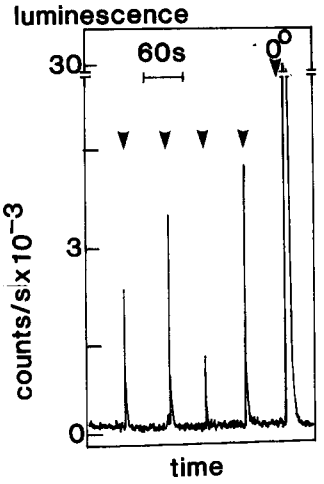


*Fig. 2.* The effect of touch stimulation on the luminescenc of tobacco seedlings transformed with the calcium-sensitive luminescent protein aequorin. One week old tobacco seedlings transformed with aequorin were placed in a luminometer and gently touched with a fine wire once every minute for four minutes (indicated by arrows). After this stimulation the seedlings were irrigated with ice-cold water (0 °C). Adapted from Knight *et al.* [36].

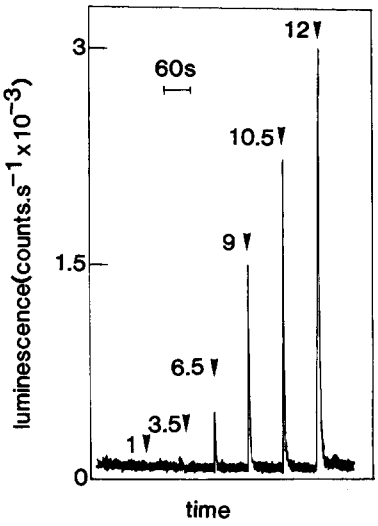


*Fig. 3.* The effect of wind stimulation on the luminescence of tobacco seedlings transformed with the calcium-sensitive luminescent protein, aequorin. A single tobacco seedling was fixed in a luminometer by the roots and stimulated by gusts of wind from a syringe. Wind forces are in newtons (N) and are indicated as numbers beside the trace. An increasing signal strength elicits an increasing luminescence. Adapted from Knight *et al.* [37].

to rock slightly (ca. 10° from vertical) about the hypocotyl/root junction causing alternate compression and tension in cells in this region. Providing the wind force exceeded a threshold value, an immediate burst of luminescent light was again observed. As the wind force increased, the seedling was observed to rock for longer periods and the detected luminescent light increased in parallel (Fig. 3). From this we concluded that while changes in compression and tension of cells continued (resulting from movement), $[Ca^{2+}]_i$ continued to rise. When the seedling stopped moving, $[Ca^{2+}]_i$ returned to the resting level.

## The source of movement-induced $[Ca^{2+}]_i$ may be intracellular

We investigated a range of calcium channel blockers, calmodulin-binding inhibitors and other inhibitors of calcium metabolism on wind-induced $[Ca^{2+}]_i$ changes [37]. Inhibitory effects were only observed with ruthenium red at low concentrations. Ruthenium red is believed to inhibit mitochondrial calcium uptake or to inhibit release of $Ca^{2+}$ from the rough ER [14]. The site of action of ruthenium red is not understood in plant cells but it is generally assumed to modify $[Ca^{2+}]_i$ release from internal stores.

## Touch and wind mechanically signal plant cells. Further evidence for the role of $[Ca^{2+}]_i$

The touch sensitivity of plants is well characterised. Early compendia by Darwin [12, 13] and Pfeffer [53] characterising and describing climbing and other touch-sensitive plants illustrate the enormous variety which climb via the agency of touch-sensitive stems, petioles, tendrils, flower peduncles and roots. While the usual response to touch is differential growth, in other cases adhesive pads or even roots may form. In a separate set of experiments, Darwin [13] described the responsiveness of roots from numerous species which bend in response to touch. Numerous insectivorous species, *Mimosa* and *Berberis* stamens

round up the list of obviously touch-sensitive plants and tissues.

Touch sensitivity is found amongst many flowering families. Thus touch sensitivity is unlikely to be a unique evolutionary adaptation arising completely *de novo* on numerous occasions but an evolutionary amplification or modification of a facility present in most or all higher plants. Climbing plants and others have simply coupled touch to an easily visible growth response. There are other numerous examples of less obvious touch-sensitive responses. There are reports in the literature that: (1) coleoptiles stroked with a cork rod bend to the stroked side [63]; (2) etiolated plants stroked with sheets of paper respond as if exposed to white light (the stems are shortened and thickened [6, 7]; (3) plant stems which are rubbed respond by synthesising ethylene and exhibit subsequent stem shortening and thickening, a phenomenon termed thigmomorphogenesis [32]; (4) stroking roots interrupts their growth for several hours [26]. Fungi are also thigmomorphogenic. *Uromyces* hyphae respond to the lip of a guard cell by forming an appressorium [28]. Pollen is also touch-sensitive and grows in defined directions on material with repetitively spaced ridges [27].

Studies in the past century with a variety of agents to touch plants led Pfeffer (quoted in [4]) to conclude that touch stimulation relies upon a localised shearing or differential deformation by the applied mechanical force. Tension and compression induced by touch and wind stimuli [36, 37] also induce localised shearing and differential deformation. *Taraxacum* peduncles held at their base were subjected to a 2 g bending stress causing about 10° bowing for five minutes [10]. Cells in the peduncle were thus subjected to tension and compression. After release from the bending stress, the peduncle continued to grow but bent away from the compressed side after a slight delay. This bowing treatment clearly mimics the compression and tension stimulus we imposed by wind stimulation on tobacco seedlings [37]. We would suggest that many touched, rubbed or stroked tissues similarly undergo slight movement and thus tension and compression during the stimulation. Transformed tobacco seedlings have been permanently bent in a luminometer and a $[Ca^{2+}]_i$ spike has been observed [36, 37].

Wind is a well recognised morphogenic stimulus [25]. In woody plants the wind-induced rocking of the stem about the roots leads to a diversion of carbohydrate resources to induce stem thickening and lignification (references in [33]). The stem becomes more resistant to sway which helps reduce further shoot damage on further episodes of wind stimulation. However the extent of thickening depends on the extent of stimulation thus rendering the character a good example of plasticity [68]. It is thought that the primary yield differences between glasshouse-grown crops and their equivalents in the field reflect the absence of wind stimulation in the greenhouse. The diversion of carbohydrate to increase stem thickening otherwise reduces the food reverse available to provision the seeds.

The effects of wind can be mimicked by intermittent shaking to sway the plant, a phenomenon called seismomorphogenesis [33]. There is a consequent reduction in height and an increase in stem thickening in both etiolated and light-grown plants [7]. Neels and Harris [47–49] reported that shaking induced premature dormancy in young trees and substantial height and yield reductions in maize.

The mechanism whereby touch and wind stimuli modify plant growth and development is not known but changes in $[Ca^{2+}]_i$ may transduce both stimuli. Aside from the measurements described above, Jones and Mitchell [33] reported that EGTA and calmodulin-binding inhibitors negated rub-induced growth reductions in soybean. Much more dramatically, Braam and Davies [5] showed that touch stimulation (mainly rubbing or stroking) of *Arabidopsis* massively induced the expression of five touch genes, three of which were identified as calmodulin or calmodulin-related proteins. These important data demonstrate that mechanical stimulation can specifically modify gene expression and again indicate a substantive role for $[Ca^{2+}]_i$.

## Plant tissues are very sensitive to mechanical stimulation

Actual figures for plant sensitivity to touch stimulation are hard to come by. Darwin reported that tendrils were sensitive to the weight of a cotton thread which he measured to be 4 mg [13]. Pfeffer reported that some tendrils were sensitive to weights of 25 $\mu$g thus making the tendrils more sensitive than human touch [53]. Only 30 s of shaking per day is sufficient to mimic wind sway and reduce height 6-fold in young trees. Clifford *et al.* showed that bowing of only 10° was sufficient to induce counteractive growth [10].

These data suggest that plant cells are extraordinarily sensitive to slight mechanical stress. It is thought that a touch stimulation is only exercised when unequal pressure is exerted at different points producing deformation of the epidermis [53]. If tissue deformation is the normal means of sensing touch the degree of deformation by a few milligrams weight on a cell wall maintained in shape by 8 atm (800 kPa) turgor must be very tiny. Deformation will be experienced first by the cell wall but information has to be conveyed to the interior of the cell if a response is to be generated. The cell wall should then be adhesively connected to both the plasma membrane and at least to the cortical cytoskeleton which is located in the non-streaming part of the cytoplasm. If these three constituents were not connected then mechanical stress would cause slippage of the cell wall over the plasma membrane and in turn over the cytoskeleton. As a consequence, cells would not maintain a specifically positioned cytoskeleton with respect to the plasma membrane. Polarized secretion leading to growth of defined regions of the wall (side walls compared to end walls for example) could not be maintained. With slippage, mechano-transduction mechanisms become more difficult to visualise.

Direct evidence supports the presence of an inter-linked cytoskeleton/plasma membrane/cell wall continuum in plant cells [72] and in animal cells [11, 29]. Studies on the fixation of polarity and localised growth in the developing *Fucus* zygote, the regeneration of differentiation initiated by protoplasting, studies on plasmolysed cells and the spatial localisation of secretion of many plant cells are some of the evidence which implies the existence of this continuum.

Some sensitive plants have been reported to contain specific thigmosensitive cells. However the simplest way to make selected tissues more sensitive is probably to increase the surface area density of mechanoreceptive constituents.

## Gravitropic sensing may be a special form of touch sensing

The commonest view of gravisensing assigns statoliths an important role. Statoliths are large, subcellular, sedimentable bodies. In higher plants statoliths are usually identified with amyloplasts and gravisensing is believed to be restricted to statocytes, specific cells which contain very prominent amyloplast structures. Sedimentation of the amyloplast onto cellular membranes is thought to initiate gravisensing. The isolation of *Arabidopsis* mutants with little or no detectable starch which are still gravitropically sensitive [9] and the rediscovery of gravity-regulated streaming rates in giant algal cells [71] has led to a reappraisal of ideas on gravisensing. These suggest that while statoliths help refine and improve gravisensing [35, 71], they may not be the absolute necessity once thought [9].

An intriguing alternative to statoliths, first proposed around the turn of the century, has recently been resuscitated [54, 71]. This suggests that gravisensing may result from detection by individual cells of the weight of the cytoplasmic bulk on the cortical cytoplasm, the plasma membrane and the appropriate cell wall – a sort of inside-out deformation or touch sensing. Calculations by Wayne *et al.* [71] show this to be a tenable hypothesis for giant algal cells but a weaker possibility for the much smaller higher-plant cell. It has also been suggested that statoliths simply increase the weight of the cytoplasm thus making the statocyte a more reliable gravitropic detector [71]. Deformation or mechanical stress is increased and this improved sensitivity explains the

widespread distribution of statocytes in graviperceptive tissues.

However sensing of cytoplasmic weight by individual higher-plant cells may not be necessary. Branches of trees which grow horizontally experience tension and compression stresses between the upper and lower parts. While the maximum stress will be experienced by the outermost cells there will be effective gradients of tension/compression throughout the tissue. Compression induces the formation of reaction wood to counterbalance the stress. Weights as little as 50 mg can induce the formation of reaction wood [20]. A horizontally placed but unsupported coleoptile or root should also experience tension and compression, with the maximum stress exerted on the upper and lower epidermal cells although of a lower magnitude than in a branch. Epidermal cells are believed to exert primary control on extension growth [39] and differential mechanical stress on these may be sufficient to initiate growth rate modification. In horizontally supported tissues, the bottom epidermal cells will still experience compression from the weight of cells above (in addition to any touch response). Based on the calculations provided by Wayne *et al.* [71] this mechanism could contribute to gravitropic sensing.

Pickard and Ding [54] have suggested that gravitropic sensing may be an evolutionary refinement of a general growth co-ordinating mechanism. Local mechanical stresses and strains occur as cells expand unevenly in growing tissues. These mechanical stresses are sensed and the subsequent fine turning of expansion rates ensures coordinated growth. It has also been hypothesised [66, 67] that this fine-tuning may be a function of auxin, smoothing out uncoordinated patterns of growth. The resulting auxin-induced cell synchronisation may then result in increased growth rates. We have recently showed that gravi-stimulating *Arabidopsis* roots leads to a 3–4-fold accumulation of calmodulin mRNA [62]. This observation again suggests a metabolic similarity between touch-induced responses [5] and gravity responses. Numerous observations relate gravitropic phenomena to calcium (e.g. [64] and references therein) deepening the possible relation to touch.

**Is there a mechanical basis to plant form?**

Plant form has a holistic basis. 'Die Pflanze bildet Zellen, nicht Zellen bilden Pflanzen' (quoted in [65]) is an important statement which summarises traditional recognition of the essential holistic character to morphogenesis. The evidence which so impressed previous generations of plant morphologists is derived from numerous experimental observations. Chief among them are the regeneration of cell differentiation and tissue patterns and finally plant form from wounded plant tissues [40]. In seeking to identify cellular characteristics from which holistic tissue properties might emerge, the most obvious is the contiguous character of tissue cell walls interacting with turgor pressure. A change in the tensile strength of the wall (or the turgor) of any one cell will inevitably have consequent effects on the shapes of surrounding cells. If larger numbers of cells change wall strength or change turgor pressure the effects will be increasingly experienced throughout the tissue-influencing form. The shape of any organ will reflect the balance of mechanical forces experienced inside it. Any of the primary tissue shapes should possess unique mechanical stress patterns resulting from inequalities of tension between the constituent cells. As growth continues these stress patterns will change. *If the mechanical stress can be sensed and transduced to specify the further directions of growth a mechanical basis to the generation of form emerges.* There is potential to specify both the primary shapes of plant organs and the positional distribution of many inside-tissues based on sensing of lines of stress through the tissue.

D'Arcy Thompson [65], like Hooke and Grew before him, was fascinated by the similarity of the shapes of parenchymatous cells to the shapes adopted by soap bubbles in a froth. Single bubbles are spherical because they assume a shape in which the surface tensions (the mechanical stresses) of the film are at a minimum. Similar

minimal mechanical stress requirements account for the shape of froth-enclosed bubbles; they would also help ensure that the surface area of interaction of the bubbles be at a minimum too.

D'Arcy Thompson [65] hypothesised that newly formed cell walls act like a fluid film and that where they join older, more rigid walls, minimum energy (stress) considerations place this new wall at an angle of 90° – a suggestion in accord with observation. As this new cell wall acquires a rigidity similar to those to which it is joined the angle of juncture assumes 120° equalising intercellular tension – an hypothesis supported by many observations.

These important observations can be used to generate simple models of form based on three premises.

1. The plane of cell division can be aligned by mechanical stress. Observations of cell division alignment induced in callus by externally applied pressure support this premise as does the alignment of cell plates induced during induction of reaction wood by compression [20, 41]. These observations suggest that mechanical stress can be sensed and transduced into rearrangements of a cytoskeletal structure specifying the plane of division.

2. The mechanical strength of individual cells walls can be different amongst the constituent cells of growing tissues. Bisection of growing sections causes the tissue to bow outwards, an observation first made in the last century. The accepted explanation for this observation is that the peripheral layers are less easily deformable than inner tissues. Peripheral cell walls are 6–20 times thicker than inner walls and evidence suggests that growing tissues are constrained by an epidermal 'straitjacket' [38]. Growing tissues contain a number of different cell types. Each cell type should have a different wall structure with different tensile strengths thus providing permanent internal tension within growing and developing tissues. The outermost epidermal wall may be the most resistant to deformation and even in 1906 was drawn as thicker than other epidermal cell walls [53].

3. The directions of growth and division be set to minimise the mechanical stress throughout the growing tissue and that a mechanism for sensing and acting upon mechanical stress be available to plant cells. This is discussed in the last section of this article.

Kutschera [38] has suggested that growing stems with their epidermal 'straitjacket' can be regarded for some purposes as like a giant turgid cell. Analogously, the spherical form of many fruits and berries can be understood as resulting from less deformable, more rigid peripheral layers surrounding a mass of inner dividing cells with softer cell walls. Conceptually this is equivalent to a bubble or a balloon providing the deformability of all epidermal cells is uniform. The spherical shape simply minimises the mechanical stress characteristics built into the tissue. Organs such as shoots and roots have a circular cross section which is the structure of minimal mechanical stress which results from the greater tension exerted by the peripheral layers. The shape is tubular rather than spherical because division and growth are constrained to the tip where wall deformability is higher. Many apical regions possess a cone-like shape and this shape can be conceived as resulting from a gradient of decreasing peripheral cell deformability as cells age. In both these organs the direction of growth is clearly specified by the directionality of tissue tension and stresses.

With an initial asymmetry built into the mechanical tension within the meristem, aspects of phyllotaxis can be described [59]. 'The behaviour of cells in the meristem is detemined not by any character or properties of their own but by the positions and the forces to which they are subject. As soon as the tension of adjacent cell walls becomes unequal then the form alters' [65]. The shoot apex in *Vinca* is not a perfect hemisphere implying a non-uniformity of tension within the apex which slowly becomes elliptical as leaves grow out [59]. The new primordial leaves thus emerge at nodal points of tension/stress within the apex. The outgrowing leaf continually modifies torsional stress within the apex until a new nodal point in tension emerges and phyllotaxis recommences. The formation and maintenance of the tunica can be understood since new cross

walls will be jointed to the thicker, more rigid outer epidermal cell wall in the growing tissue and thus remain at an angle of 90° [65]. Circummutation is perhaps a later indication of an earlier asymmetry of growth in the apex.

An initially tubular primordial leaf can be turned into a flat blade by limited spatial strengthening of the peripheral walls on top and bottom of the tissue; dividing cells are literally squeezed out at either side where there is less resistance to division and growth. In other primordial leaves which are effectively flat at birth, localised peripheral layer strengthening can again account for the form. The production of form becomes a kind of structural epigenesis in which the sensing of tension and compression followed by adjustments of growth direction become critical elements.

Mechanical hypotheses explaining the production of form are attractive because of the simplicity of mechanism they offer [51]. These hypotheses have gone largely unrecognised because of a lack of appreciation that plants can sensitively perceive mechanical stress and more importantly can act upon it. However mechanical bases to the production of form have been championed for many years by Paul Green (see [59]) and, more lately, by Brian Goodwin and coworkers [23, 24]. There has also been a dominance of this topic of investigation by notions of chemically specified positional information, a still unsubstantiated theory [51]. No doubt aspects of both mechanisms contribute.

## How are mechanical signals sensed? The role of calcium

Predominant interest in animal cells centres upon mechanical sensing by integrins [11, 21, 29, 30]. These data will be discussed since it is most likely that equivalents exist in plant cells as considered later.

Integrins are plasma membrane-spanning proteins composed of two separate subunits. Regions of the integrin molecule penetrate the extracellular matrix where they bind to fibronectin, vitronectin and laminins and perhaps other extracellular adhesive molecules. The cytoplasmic face of the integrin molecule acts as a nucleation or anchorage site for the attachment of cytoskeletal structures through the linking proteins vinculin, talin and actinin. At least 19 different integrins have been characterised so far and it seems likely that each may perform slightly different functions in mechanical signalling or be located in different cell types; some act to nucleate microfilaments and some intermediate filaments for example.

Many motile animal cells possess focal adhesion sites through which they attach themselves to the substratum. Integrins are specifically concentrated in focal adhesion sites [8]. Here they act as a focus for the formation of a cytoskeletal network (so-called stress fibres) which spreads from these sites throughout the cell. The attached cytoskeletal network is under tension and this provides shape or form to the cell. If the focal adhesion sites are disrupted, cells will round up and become quiescent. Integrins therefore directly mediate mechanical stress between the extracellular matrix and the cytoskeleton.

A direct role for integrins in sensing mechanical stress was demonstrated by Wang *et al.* [70]. Vitronectins have a conserved peptide region, notably the sequence RGD (Arg-Gly-Asp), which binds to integrins and can be used to probe integrin function. Tiny ferromagnetic beads were coated with RGD peptide and allowed to adhere to endothelial cells where they attached themselves to the exposed face or integrin molecules. On attachment the beads initiate integrin clustering, the step believed to induce signalling. Talin, vinculin and actinin were observed to be recruited into the region of bead attachment indicating the formation of microfilaments attached to the clustered integrins. A magnetic force was then applied to twist the bead and mechanically stress the attached microfilaments. Surprisingly, integrin-attached microfilaments showed increasing stiffness (ratio of stress to strain) in direct proportion to the applied stress. In fact the bead could only be rotated by 25°. By using appropiate inhibitors the authors demonstrated that the microfilaments had become attached to, and become part of, a cytoskeletal network stretching through-

out the cell. This network continually rearranges as stress is increased in an attempt to resist further torsional stress. Mechanotransduction may thus be mediated simultaneously at multiple locations inside the cell through force-induced rearrangements of an integrated cytoskeleton.

Integrin clustering and thus signalling can be induced by anti-integrins. Integrin signalling increases $[Ca^{2+}]_i$, changes phosphatidylinositol (PI) metabolism and membrane protein phosphorylation. There are specific alterations in gene expression. Since nuclei are sometimes held in a basket of mirofilaments, restructuring of the cytoskeleton after integrin signalling may directly modify chromatin structure and thus gene expression. However in one well-characterised case it is nuclear $[Ca^{2+}]_i$ which is specifically increased [61]. Altered gene expression may then directly result from combination with nuclear calmodulin and altered nuclear calmodulin dependant protein kinase activity. Increases in $[Ca^{2+}]_i$ may also act to promote microfilament reconstruction through activation of gelsolin issuing from force-induced cytoskeletal rearrangements. Since mechanical stress can modify the plane of division [41], integrins may be involved in directing the position of the preprophase band of microtubules.

Recent exploration of integrin signalling has indicated that intracellular events can also influence the conformation and ligand binding affinity of the extracellular domain of integrins. This 'inside-out' modification of signal transduction appears to be mediated through the cytoplasmic domains [21]. The integrin surface repertoire is altered, enormously increasing the range and affinity for different ligands. Thus internal changes can determine which components of the extracellular matrix are recognised and modify the sensitivity to mechanical stress. This 'inside out' signalling is reminiscent of the way in which gravitropic stimulation might be an inside-out version of the touch response.

Are there integrins in plant cells? Present indications are tantalising. Sequences of integrins are not highly conserved so direct sequence equivalents are unlikely [72]. However vitronectin-like molecules have been detected in numerous plant and even algal species [22, 57, 69]. Vitronectin along with fucoidan is concentrated in the growing rhizoid wall of the early *Fucus* zygote. If this vitronectin acts as attachment sites for integrin-like molecules and microfilaments inside the cell this would help explain the localised secretion of polysaccharide-containing vesicles which feed the growing wall. Vitronectins are part of the adhesion mechanism used by the early zygote to attach itself to a substratum. In the absence of adhesion the rhizoid fails to develop and instead the zygote becomes a ball of undifferentiated cells. Adhesion may be an unrecognised but important aspect of plant morphogenesis. Schindler *et al.* [58] and Wyatt and Karpita [72] have used the RGD peptide to inhibit vitronectin binding by integrins in plant cells. In both cases modified adhesion of the plasma membrane to the cell wall was observed.

There is no reason to suppose that mechanical stress sensing is confined to integrins in plant cells. The wall is compositionally complex and theoretically any of the primary wall constituents could bind to an appropiate and specific plasma membrane-spanning protein that could be used for mechanosensing. Indications that wall components are sensed may be deduced from the known effects of oligosaccharides in defense reactions and cell development [72]. Polysaccharides secreted by one cell could promote cytoskeletal rearrangements in adjacent cells thus leading to phenomena such as homeogenetic induction [40].

The role of calcium in mechanical sensing and transduction has yet to be well defined but a function in microfilament rearrangement via gelsolin is one good possibility. Kirchofer *et al.* [34] demonstrated a very direct requirement for calcium ions for integrin attachment to vitronectin and thus for adhesion. A role for plant cell wall calcium is thus suggested in addition to pectin binding. Goodwin and collaborators [23, 24] have suggested that the cortical cytoskeleton is an integrated visoelastic network under tension whose properties are directly modified by the free $[Ca^{2+}]_i$. They have suggested that nodal stress points appear in this network at critical $Ca^{2+}$ concentrations which could then be used to

specify local areas of secretion of wall-softening enzymes. They demonstrate that their mechanism could explain aspects of morphogenesis in *Acetabularia*. Their ideas are in part based on equivalent mechanical signalling mechanisms proposed to underlie animal morphogenesis [51, 52]. Ding and Pickard [15] have characterised stretch-activated calcium channels in onion epidermal cells which cluster in opening activity. Stretch channels are an alternative means for signalling mechanical stress but may require a significant change in membrane area before activation. Ding and Pickard [15] have suggested these stretch channels might be directly connected to integrins suggesting a very basic role for such channels in signalling.

## Conclusions

This short article has illustrated aspects of mechanical sensing and transduction in plant cells. The importance of mechanical stress in the production of form has almost certainly been underestimated and hopefully a resurgence of interest will clarify the relevance to morphogenesis. There is increasing attention being paid to the plasma membrane/cytoskeleton/cell wall continuum and this should help clarify the role of mechanical stress. The significance of $[Ca^{2+}]_i$ to mechanical signalling is not yet clear but circumstantial evidence strongly suggests it is an important component of the transduction process. The next step is to identify the origins of mechanically induced changes in $[Ca^{2+}]_i$ and the mechanism whereby increases occur. These are in hand.

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