A UNIFIED HYPOTHESIS OF MECHANOPERCEPTION IN PLANTS

FRANK W. TELEWSKI

W. J. Beal Botanical Garden, Department of Plant Biology, Michigan State University, East Lansing, Michigan 48824 USA

The perception of mechanical stimuli in the environment is crucial to the survival of all living organisms. Recent advances have led to the proposal of a plant-specific mechanosensory network within plant cells that is similar to the previously described network in animal systems. This sensory network is the basis for a unifying hypothesis, which may account for the perception of numerous mechanical signals including gravitropic, thigmomorphic, thigmotropic, self-loading, growth strains, turgor pressure, xylem pressure potential, and sound. The current state of our knowledge of a mechanosensory network in plants is reviewed, and two mechanoreceptor models are considered: a plasmodesmosome-based cytoskeleton–plasma membrane–cell wall (CPMCW) network vs. stretch-activated ion channels. Post-mechanosensory physiological responses to mechanical stresses are also reviewed, and future research directions in the area of mechanoperception and response are recommended.

Key words: gravitropism; gravity; mechanoperception; sound; thigmomorphogenesis; thigmotropism; turgor pressure; wind.

The ability to sense and respond to physical stimuli is of key importance to all living things. Among the common environmental stimuli detected by living organisms are light, temperature, and a variety of chemical signals. A number of these stimuli appear to be closely related and can be considered as physical–mechanical stimuli, that is differences in a mechanical force or pressure perceived by the living cell. A cell may perceive gravity; strains caused by self-loading and internal growth; mechanical loading by snow, ice, and fruit, wind, rainfall, touch, sound; and the state of hydration within a cell (turgor pressure). All organisms appear to perceive these mechanical signals, regardless of their taxonomic classification or life habit (sessile vs. motile). The significant differences between taxonomic groups, specifically plants and animals, are found in the individual molecular components of the microstructure of the internal cellular sensing network (Jaffe et al., 2002; Baluška et al., 2003) and in the response of an individual organism to each mechanical stimulus.

Internal mechanical forces—The sensing of gravitropic signals by plants has been studied for 200 years (Knight, 1806). Since the first study, the elucidation of the mechanism of gravitropism has been researched in a broad array of plants from algae to trees and in a variety of plant organs. To date, two compelling hypotheses exist regarding graviperception in plants: the starch–statolith hypothesis and the hydrostatic model of gravisensing (for reviews, see Sack, 1991, 1997; Baluška and Hasenfus, 1997; Staves et al., 1997; MacCleery and Kiss, 1999; Boonsirichai et al., 2002; Drobot et al., 2004). Both hypotheses ultimately rely on the sensing of a mechanical signal at the cytoskeleton–plasma membrane–cell wall interface (CPMCW) interface. In the case of statoliths, falling starch grains or other organelles impact the plasma wall interface (CPMCW) interface. In the case of statoliths, the reorientation of a plant organ within a gravitational field is proposed to induce internal pressure differences at the CPMCW interface, which can be considered an external mechanical signal (Staves et al., 1997; MacCleery and Kiss, 1999; Baluška et al., 2005). Therefore, a more broadly unifying mechanism may underlie gravisensing in plants than that evoked by a hypothesis that relies on how the mechanical signal is initiated; an actual sensory structure within the cell may allow for mechanoperception as the plant is reoriented with respect to gravity. Supporting the concept of a unified hypothesis for mechanical sensing in the gravitropic response is the work of Massa and Gilroy (2003) who reported when a root cap came in contact with a horizontal glass plate (inducing thigmotropic stimulus), the root cells behind the growing tip began to grow horizontally. This allowed the root cap to maintain contact with the plate, while the rest of the root grew over and parallel to the obstacle with a step-like growth form. The authors suggested that the gravisensitive cells of the root cap also sense the touch and signal the columella cells to alter their gravitropic response, so that they act together to redirect root growth to avoid obstacles while continuing a general downward pattern of growth.

In plants, gravitropism can occur in either primary or secondary tissues. In primary growth, the gravitropic curvature results from differential cell elongation on opposite sides of the displaced organ. In the case of secondary growth, the gravitropic response includes the formation of reaction wood; tension wood in porous angiosperms and compression wood in nonporous angiosperms and gymnosperms (Timell, 1986a). Tension wood forms on the upper side of a displaced stem and is characterized by the formation of gelatinous fibers with lower lignin content, smaller diameter, and fewer vessels and by a realignment of cellulose microfibrils into a vertical orientation within the gelatinous layer, which forms inside a partially developed and lignified S2 layer of secondary cell walls of gelatinous fibers. Compression wood occurs on the lower side of a displaced stem and is characterized by the formation of tracheids with a thickened secondary cell wall with higher lignin content, a round cross section, intracellular spaces at cell corners, and a realignment of cellulose microfibrils in the S2 layer to a 45° to 60° orientation with respect to the axis of the stem.

The formation of reaction wood in stems, branches, and roots is not an exclusive response to gravity in woody plants. The formation of reaction wood has also been observed to
develop in branches and stems as a means of reshaping crowns and as a possible phototropic response (Engler, 1924). Tension wood has been reported to form in the vertical stems of rapidly growing poplar (Populus) trees (for a review, see Telewski et al., 1996). Timell (1986c) suggested that the reaction wood may form to keep woody plants in balance with their physical environment (e.g., gravity, wind, and light), subsequently generating internal growth strains that result in the physical reorientation of woody plant organs.

The maturation of xylem cells in the cambial zone involves the alteration of individual cell lengths. In many instances, there is intrusive growth in which the cells elongate within the relatively rigid structure of the stem, inducing internal compressive forces (Boyd, 1985; Archer, 1987; Fournier et al., 1991a, b; Larson, 1994). In other cases, the cells shrink upon maturation inducing a tensile force within the stem. The generation of these internal growth strains is responsible for the realignment of stems in the gravitropic response, with compression wood developing a compressive growth strain and tension wood forming a tensile growth strain (Wilson, 1981; Alméras et al., 2005). Growth strains also develop in stems aligned vertically with respect to gravity and may function to maintain mechanical balance within woody plants as part of a phototropic response, self-loading, or from differential loading caused by crown asymmetry (Archer, 1987).

Within a vertically aligned stem, there are two potential sources of compressive force loading. The most obvious is due to self-loading along the vertical axis of the stem as a result of the accelerating force of gravity. A second compressive force has been suggested to be induced by the constrictive nature of bark tissues (referred to as bark pressure), resulting in a radial compressive force that affects xyleogenesis in the cambial zone (DeVries, 1875). In earlier studies, the radial compressive force of a constricting outer bark was hypothesized to increase during the growing season from the radial growth of the cambium and to be responsible for the formation of smaller, denser lateward cells and the ultimate formation of annual growth rings (for a review, see Larson, 1960). In subsequent studies, this hypothesis was refuted, and annual growth rings were found to form in response to external environmental stimuli including day length and changes in plant growth regulator content (for review, see Little and Savidge, 1987; Roberts et al., 1988; Larson, 1994). Although the bark pressure hypothesis appears to bear little on the formation of annual growth rings, the application of a compressive force to cambial explants (tissue culture) appears to function in maintaining the structure and organization of the vascular cambium in vitro, ensuring the continued production of apparently normal xylem (Brown and Sax, 1962; Brown, 1964; Makino et al., 1983).

Additionally, self-loading along a vertical axis contributes significantly to development in plants. The ability of a tree to perceive its own weight must play a significant role in determining overall allometry and mechanical properties of wood (density and elastic modulus) produced by a mechanically loaded vascular cambium, in the absence of any lateral loading induced by wind or other external mechanical forces (McMahon, 1973; Wainwright et al., 1976; Niklas, 1992, 1994). In a few studies, the application of a compressive force induced differentiation of cambium initials within a mass of dedifferentiated callus cells and within graft unions (Lintilhac and Vesecky, 1981; Barnett and Asante, 2000). The notion that self-loading will impact the formation of a vascular cambium and subsequent secondary xyleogenesis has recently been further supported by a study on wood formation in Arabidopsis in which the application of weight, and thus a compressive force on the stem, induced secondary growth in a species that only produces herbaceous growth under “normal” environmental conditions (Ko et al., 2004). Ko et al. suggested that the mechanical stimulus of self-weight is perceived by the stem and induces the differentiation of a secondary vascular cambium and subsequent formation of secondary xylem and that self-weight may play a more important role in the development of the woody plant growth habit.

The self-loading induced by the bearing of fruit has been shown to influence growth in branches (Alméras et al., 2004; Vaast et al., 2005). The forces associated with the bearing of fruit are primarily perceived in branches and at branch bases and consist of an alternate compressive force on the lower side of the branch and a tensile force on the upper side of the branch so that the branch acts like a cantilever (Wainwright et al., 1976; Niklas, 1992). The additional load will stimulate increased reaction wood formation in the branches.

Each living cell within a plant, with its organelles and protoplasm, functions mechanically like a water-filled balloon (hydrostat), exerting a circumferential tensile force and radial compressive force within the plasma membrane and pressing against the surrounding cell wall. The plasma membrane controls turgor by regulating the flow of water and solutes between the apoplast and symplast. Turgor pressure can be increased (hypertonic) or decreased (hypotonic) by altering the osmotic potential of the apoplast or symplast, thus either forcing water into a cell increasing turgor or by drawing water out of a cell and decreasing turgor (plasmolysis). Turgor is also decreased by drought stress, sometimes resulting in the loss of mechanical strength of plant tissues, which results in wilting. Under these conditions, turgor pressure can contribute significantly to the mechanical properties of plants, especially in soft, non- or low-lignified tissues and organs characteristic of primary growth (for reviews, see Niklas, 1989, 1991). Sensing changes in turgor is crucial to survival in plants. It is possible that changes in turgor impart mechanical stresses on the CPMCW, which serves as the mechanosensory network for plant cells. Sensing water potential within the plant via internal mechanical stresses at the cellular level could be the means for what is termed hydraulic signaling, providing a faster signaling and stomatal response to the onset of drought stress than could be predicted by a root-generated chemical signal such as abscisic acid (Comstock, 2002). Hayashi et al. (2006) provided data to support the role of both the CPMCW and Ca2+-mechanosensitive stretch-activated ion channels in plant cells under hypotonic and hypertonic conditions. They report stretch-activated ion channels function in sensing both hypotonic and hypertonic conditions, where as the CPMCW is only involved in sensing hypertonic conditions.

**External mechanical forces**—Numerous external mechanical stimuli can be perceived by an organism. Some are induced by gradients in pressure within the atmosphere and are responsible for wind, while pressure gradients in aquatic systems are created by currents or tidal flows. Pressure waves that form sound waves are transmitted in both aerial and aquatic environments. Other mechanical stimuli are induced by gravity, such as the accumulation of ice or snow, but do not necessarily induce a gravitropic response. A third category can be classified as touch, such as that induced by the impact of raindrops, hailstones, other inanimate objects, or by other...
organisms. These mechanical stimuli have collectively been termed touch or thigmomorphogenic and produce a number of thigmomorphogenetic responses in plants, including thigmomorphogenesis, thigmotropism, thigmomancy, and the thigmotactic response (Jaffe et al., 2002; Braam, 2005). Once again, the perception of an external touch or mechanical signals depends upon the CPMCW mechanosensing network.

Vogel (1994) outlined the parameters influencing life in moving fluids and provided the fundamentals needed to understand the physics of fluid dynamics in aquatic systems and the atmosphere. As mentioned previously, differences in atmospheric pressure result in pressure waves defined as currents of air or wind. Currents in aquatic ecosystems are also pressure waves. Waves in aquatic systems or wind can be laminar or turbulent (Vogel, 1994). Due to the greater density and viscosity of water, the force imposed by a wave at a given velocity on a structure is much greater than by wind. Denny and Gaylord (2002) gave the example of a 2 m $\cdot$ s$^{-1}$ (4.5 miles $\cdot$ h$^{-1}$) velocity wave as being roughly equivalent to a 58 m $\cdot$ s$^{-1}$ (130 miles $\cdot$ h$^{-1}$ or between a category 3 and 4 hurricane) wind in terms of applied force. They go on to state that 25 m $\cdot$ s$^{-1}$ wave velocities are not uncommon in shoreline environments and that such a wave exerts the equivalent force of that nearly equal to a wind in excess of Mach 2. One of the complexities of studying the response of plant mechanical stresses imposed by wind or wave is disecting the individual forces acting upon the structure of a plant. Wind places an asymmetric pressure on the side of a plant creating a cantilever with the rotation point located in the root plate (Vogel, 1994). Wind induced sway is considered the primary mechanical stress inducing an alternating compressive and tensional force, with some torsion applied in stems and roots (Telewski, 1995). Pressure waves can also displace the stem within the gravitational field long enough to induce a gravitropic response, which should be considered as a secondary wind-induced mechanical stress (Telewski, 1995). Due to the neutrally buoyant nature of aquatic systems, macrophytes may not require a significant gravitropic response upon displacement by waves. The weak and highly compliant stems of large algae allow for them to be highly flexible rather than protective and rigid like the stems of most terrestrial plants (Denny and Gaylord, 2002). This highly flexible structure allows algae to float back to the vertical orientation when wave action ceases, without the need to induce specialized strain-generating tissues such as reaction wood that is needed to re-orient a terrestrial plant within a gravitational field.

The ability of a plant to respond to wind (for reviews, see Jaffe, 1985; Biddington, 1986; Vogel, 1994; Telewski, 1995) or waves (Vogel, 1994; Koehl, 1999; Puijalon and Boremette, 2004; Puijalon et al., 2005) by altering morphology, anatomy, and biomechanical properties enables the plant to withstand additional mechanical loading. People have long observed that wind influences the morphology and growth of plants, especially trees, creating metaphors and lyrics about trees growing in windy environments being “tougher” and able to endure hardship. The first scientific study to document the influence of wind on tree growth was published by Knight (1803) in which staked apple trees (Malus) produced less radial growth than trees allowed to sway freely in the wind. When Knight restricted wind-induced motion to the bilateral (north to south), the stem formed an oval with the proportion of 13:11. Knight went on to state (p. 281):

If a tree be placed in a high and exposed situation, where it is much kept in motion by winds, the new matter which it generates will be deposited chiefly in the roots and lower parts of the trunk; and the diameter of the latter will diminish rapidly in its ascent. . . . the growth of the insulated tree on the mountain will be, as we always find it, low and sturdy, and well calculated to resist the heavy gales to which its situation constantly exposes it.

The alteration in growth and stem allometry in response to wind first described by Knight as an increase in radial growth and a decrease in height growth would be defined 170 years later by Jaffe (1973) as thigmomorphogenesis and later as the thigmomorphogenetic theory (Jaffe, 1984). This term is now used to describe the response of plants to wind and other mechanical perturbations, including mechanical bending or flexing or by touching or brushing by passing animals. Similar to the topic of gravitropism, the intervening 203 years since the printing of Knight’s thigmomorphogenetic study have seen a multitude of papers reporting the influence of wind or other mechanical perturbations on plant growth, many of them summarized in the reviews by Grace (1977), Jaffe (1984, 1985), Biddington (1986), Vogel (1994), Telewski (1995), Mitchell (1996), Jaffe et al. (2002), and Braam (2005). The perception of a thigmomorphogenetic stress by the mechanosensing network is rapidly followed by a mechanoresponse cascade, which has been shown to be dose dependent (Ermer et al., 1980; Jaffe et al., 1980; Braam and Davis, 1990; Knight et al., 1992; Telewski et al., 1997; Telewski and Puyrin, 1998; Hepworth and Vincent, 1999; Telewski, 2000). The mechanophysiological-response cascade will be addressed later in this manuscript. Characterization of the forces within a bending or flexing plant organ can be difficult to characterize and quantify due to the differing nature of the applied force, geometry, and morphology of the organ and the anisotropic nature of different tissues, which comprise the plant organ as a cellular composite material (Niklas and Moon, 1988; Beusmans and Silk, 1988; Niklas, 1992; Vogel, 1992; Mouila et al., 1994; Mouila and Fournier, 1997; Coutand et al., 2000; Coutand and Mouila, 2000; Telewski, 2000). In a detailed analysis of the mechanical stimulus of bending and resulting growth response in tomato (Lycopersicon esculentum Mill. Var. VFN8), Coutand and Mouila (2000) reported that mechanosensing is both local and scattered through the stem and explained the variability of the growth response by the integrals of the longitudinal strain field within the bending stem. Jaffe et al. (1980), Telewski and Puyrin (1998), and Coutand and Mouila (2000) reported a logarithmic relationship of the sensory function between the dose of mechanical stimulus and growth response. Coutand et al. (2000) reported that increasing the force applied to tomato stems from 0 to 175 g or stem displacement from 10° to 25° did not influence the duration of the growth response. Therefore, the dose response appears to be sensitive to the number of perturbations and not sensitive to the amount of force applied in each individual perturbation (Coutand et al., 2000).

By definition, sound is acoustic energy in the form of an oscillatory concussive pressure wave transmitted through gases, liquids, and solids. It is audible to the human ear and falls into the frequency range of 20–2000 Hz. Sound above this range is classified as ultrasound, and sound below this range is infrasound. One only needs to hold one’s hand in front of a base speaker or be next to a car in which the volume is turned up to feel the pressure wave in the near infrasound range.
Vegetation is known to absorb acoustic energy (Eyring, 1946; Martens and Michelsen, 1981; Price et al., 1988) and has been employed to deaden the noise of urban environments (Huisman and Attenborough, 1991; Attenborough, 2002). However, unlike wind or waves, the level of sound energy normally experienced by plants in the environment does not appear to invoke a significant compromising mechanical stress to plant structure. Mechanical energy imparted to a plant stem can induce it to sway to its resonant frequencies, usually in the infrasound range, which will be a function of the height and mechanical properties of its tissues. These multiple resonance frequencies can be used, in turn, to calculate the height, flexural stiffness, and modulus of elasticity of the stem (for a review, see Niklas and Moon, 1988). As discussed, plants need to perceive and respond to wind or wave induced movement to acclimate to a given environment. They will even sway in a harmonic motion at low resonant frequencies. Although plants can effectively absorb sound and even generate sound via wind-induced resonance of various structures such as needles and spines (for example, the whispering winds in a pine forest), can they perceive sound, and, if perceived, will they respond to sound? Is there a developmental advantage to responding to sound leading to acclimation to sonic stresses in the environment?

Ultrasound has been shown to have the greatest effect on plants, specifically on seed germination (for reviews, see Davidov, 1961; Timonin, 1966; Halstead and Vicario, 1969; Hageseth, 1974; Weinberger and Burton, 1981; Miyoshi and Mii, 1988). Timonin (1966) reported that ultrasound treatment altered the viscosity of macromolecule solutions in seeds. Near ultrasound (1.4 kHz, 0.095 kdb) was reported to increase metabolism in chrysanthemum roots, characterized by increases in amylase activity, soluble sugar, and protein (Yi et al., 2003). The treatment of chrysanthemum callus with 1.4 kHz sound increases indoleacetic acid levels while decreasing abscisic acid levels (Wang et al., 2004).

The perception and response of plants to sound, more specifically music, has been a part of folklore (see Weinberger and Graefe, 1973) and the source of inspiration for countless primary and secondary school student science fair projects beginning in the 1940s (Klein and Edsall, 1965; personal observation). The influence of music, a complex mixture of notes, tones, amplitudes, and harmonics, on plant growth has been the subject of scientific debate for decades. Singh and Ponniah (1955a, b, 1963) reported on the stimulatory influence of music on plant growth in a number of species. Klein and Edsall (1965) reported no influence of a diverse selection of music, from classical to rock and roll, on the growth of Tagetes erecta L. Weinberger and colleagues conducted a number of studies on the influence of both music and single frequency sound, both in the audible and ultrasound range, on plant growth and seed germination and reported that sound can influence plant growth (Weinberger and Measures, 1968; Measures and Weinberger, 1970; Weinberger and Das, 1971; Weinberger and Graefe, 1973; Weinberger et al., 1979; Weinberger and Burton, 1981). Most recently, Creath, and Schwartz (2004) reported music increased the rate of seed germination in zucchini (Cucurbita pepo L.) and okra (Abelmoschus esculentus (L.) Moench).

Touche, defined as the act of making physical contact with another solid object and inducing a mechanical stimulus, leads to two other thigmoresponses in plants (for reviews, see Jaffe et al., 2002; Braam, 2005). This group of two responses to mechanical stimuli includes one of the most dramatic responses in the plant kingdom, thigmomonic movements, most commonly associated with the rapid movement in plants in response to touching. Numerous plant traps are thigmotropic in nature and were described by Darwin (1893). These include the Venus’ flytrap (Dionaea muscipula Ellis ex L.), first described by Curtis (1834), which produces a trap from a modified leaf. On the abaxial surface of each half of the leaf traps are three mechanosensing trigger hairs, which, when stimulated, close the trap within a second (Brown, 1916). The movement by the modified leaf trap and the tentacles of the sundew (Drosera rotundifolia L.) incorporates both thigmotonic and thigmotropic responses (Lloyd, 1942). Darwin (1880, 1893) reported that the force imparted to the tentacles covering the surface of the leaf trap by the weight of a human hair was sufficient to supply the mechanostimulus to induce a response, yet neither wind nor rain triggered a response. The sensitive plant (Mimosa pudica L.) also has a rapid thigmotropic response with the rapid folding of its leaflets and movement of the entire compound leaf at the pulvinus upon touch. Similar thigmotropic responses have been reported in Blopytum sensitivium (L) DC (syn. Cassia sensitivum L.) and in certain Oxalis species (Umbrath, 1958).

Thigmotropic movements, induced by unilateral contact with another living organism such as a pollinator or mechanical structure, result in alterations in plant growth that include the bending of floral parts towards pollinators, the twining of stems or roots for physical support, and the coiling of tendrils (for review, see Jaffe et al., 2002).

**Structure of the mechanosensing network in plant cells**—Morris and Homann (2001) reviewed the concepts of cell surface area regulation and membrane tension, providing insight into the role of membrane tension in cell biomechanics and the potential role of membrane tension in mechanoperception in both plants and animals. To provide scale, the resting tension of a plant protoplast membrane was reported to be 0.12 mN · m⁻¹ (Kell and Glaser, 1993), the force required to activate mechanosensitive channels is approximately 1 mN · m⁻¹ (Sachs and Morris, 1998), and the lytic tension for plant protoplasts is 4 mN · m⁻¹ (Kell and Glaser, 1993). The stretching and relaxation of the cell membrane in response to changes in the mechanical environment of cells as a component of mechanosensing fits well with earlier reports of the role of stretch-activated membrane channels in the response of plants to mechanical stresses (Edwards and Pickard, 1987; Ding and Pickard, 1993). The perception of a mechanical signal by cells is a rapid process with a rapid translation of the mechanical force into a biochemical or bioelectric message (Baluška et al., 2003; Ingber, 2003a, b). Significant progress has been reported in the elucidation of the molecular basis of mechanosensory perception and transduction in animal systems, particularly the physical coupling between the cytoskeleton and cell membrane, which provides a continuous structural/mechanical network throughout the cell (for reviews, see Janmey, 1998; Gillespie and Walker, 2001; Baluška et al., 2003; Ingber, 2003a, b). Jaffe et al. (2002) proposed that a similar mechanosensing network exists within plant cells, linking the cytoskeleton–plasma membrane–cell wall structures. Their model proposed linker molecules within plant cells, as RGD (arg- gly-asp)-containing peptides, similar to the integrins, RGD-containing proteins in multicellular eukaryotes that lack a cell wall. In non-plant and non-fungal systems, the integrins facilitate bidirectional signaling and bind to actins (for review
see Baluška et al., 2003). In plants, the RGD integrin-like peptide linkages were proposed to connect microtubules to the plasma membrane, which contains Ca^{2+} ion channels. Hechtian strands [plasma membrane sleeves containing endoplasmic reticulum, actin microfilaments, and microtubules (Lang et al., 2004)] then bind the plasma membrane to the cell wall via the actin-binding, integrin-like peptides attached to the interior of the membrane, which facilitated the opening and closing of stretch-activated membrane channels (Jaffe et al., 2002).

Baluška et al. (2003) compared and contrasted the linker molecules between the cytoskeleton and cell membrane in animal and plant systems. They argued that the failure to find true integrin homologs in plants or fungi precluded their role in the plant or fungi mechanosensing network (Hussey et al., 2002) and that plants may have their own unique set of actin-binding proteins. Among the molecules functioning as linkers between the cytoskeleton and cell wall, proposed by Baluška et al. (2003) in their model, are cell-wall-associated kinases (WAKs), pectins, arabinogalactan proteins (AGPs), cellulose synthase, formins, plant-specific myosins of class VIII, phospholipase D, and callose synthase. In the past three years since Baluška et al. (2003) proposed their model of a mechanosensing network linking the CPMCW at cross-walls, further research indicates that plant-specific myosins of class VIII and formins are the strongest candidates as the elusive adhesive molecules (Baluška and Hlávka, 2005). Cross-walls, located at the nongrowing axial end of cells are enriched with plasmodesmata, actin, myosin VIII, and profilin (reviewed by Baluška and Hlávka, 2005). Recently, two studies confirmed the role of formins in nucleation and bundling of F-actin (Michelot et al., 2005; Yi et al., 2005). Deeks et al. (2005) reported that the cross walls of roots, hypocotyls, and shoot cells of Arabidopsis were rich in group 1 formins (proteins AtFH4 and AtFH8) and that AtFH4 binds to profilin, influencing the polymerization of actin. Strengthening the putative role of formins as adhesive molecules linking the cell wall and cytoskeleton is the extensin-like domain of the group 1 formins predicted to insert into the cell wall (Crvčková, 2000; Crvčková et al., 2004).

Myosin VIII, also located at cross walls and bound to actin filaments within the cytoplasm, is reported to be involved in callose synthesis, possibly binding with a membrane-spanning callose synthase subunit and providing a callosic crosslink between the cell wall and cytoskeleton at cell plates, pit fields, and plasmodesmata (Baluška et al., 2005; Baluška and Hlávka, 2005). Lang et al. (2004) reported callose was localized along the fibrous meshwork covering Hechtian strands, Hechtian reticulum at the cell wall, and protoplast 4 h after plasmolysis. Although the meshwork fibers joined the cell wall, Lang et al. (2004) failed to observe callose directly on the plasma membrane. Callose synthesis is associated with a number of wound, thermal, and mechanical stress responses as well as with fungal attack and pollen tube elongation. Jaffe and Telewski (1984) reported callose deposition increased in the phloem of bean (Phaseolus vulgaris L.) and loblolly pine (Pinus taeda L.) stems 1 h after mechanical stimulation, peaking after 9 h, and was completely reabsorbed by 25 h. Jaffe and Leopold (1984) similarly reported callose deposition within 5 min of gravity stimulation in response to gravity in Zea mays L. and Pismum sativum L. Callose and laricinan, which is similar to callose, are components of compression wood cell walls (Hoffmann and Timell, 1970). The deposition of callose inhibits cell-to-cell communication at plasmodesmata (Sivaguru et al., 2000). These studies support a role for callose synthesis in mechanoperception.

The complex structure and function of the actin cytoskeleton in plants and its linkage to the cell membrane and cell wall continues to be elucidated. One of the roles appears to be in endocytosis as well as in signaling (Šamaj et al., 2004, 2005). Baluška et al. (2005, p 106) proposed that the combined role of the actin cytoskeleton in both signaling and endocytosis, combined with the actin- and pectin-rich adhesive domains of cross walls at the polar ends of cells, comprise a “...plant developmental synapse” in which auxin and pectin-derived signaling molecules act as plant-specific transmitters for cell-to-cell communications.” In their review of cell surface area and membrane tension, Morris and Homann (2001) reported that high tension of the cell membrane promotes exocytosis and low tension promotes endocytosis. These observations lead Baluška et al. (2005) to further propose a mechanical mechanism to explain the perception of a gravitational signal in plants. Displacement of a plant cell from the vertical position alters the gravitational loading imposed upon its plasma membrane, increasing tension or stretching the upper side of the plasma membrane and decreasing tension on the lower side. This, the authors propose, will shift the position of the synaptic domains that secrete auxin, resulting in the observed accumulation of auxin at the bottom of the displaced cell (Friml et al., 2002; Ottenschläger et al., 2003) and the induction of a gravitropic response.

Baluška et al. (2003) never included a role for stretch-activated membrane channels in their mechanosensing network model. A second model for mechanosensing, based on the adhesion of the cell membrane to the cell wall involves arabinogalactan proteins (AGPs), and wall-associated kinase (WAK), does include stretch-activated channels (Ding and Pickard, 1993; Pickard and Fujiki, 2005). Gens et al. (2000) provided evidence from BY-2 tobacco cells that mechanosensory (stretch-activated) calcium-selective cation channels are grouped on the cell membrane and are associated with AGPs and WAKs, which appear to fasten the cell membrane to the cell wall. The grouping of these molecules and channels was termed the plasmalemmal reticulum (Gens et al., 2000). Mechanosensory calcium-selective cation channels also have been reported in the guard cells of Vicia (Cosgrove and Hedrich, 1991) and lily pollen tubes (Dutta and Robinson, 2004); however, the plasmalemmal reticulum has only been observed in the BY-2 cells (Pickard and Fujiki, 2005). The putative role of WAKs and AGPs in the cytoskeleton-plasma membrane-cell wall continuum and mechanosensing was reviewed by Baluška et al. (2003).

**Physiological memory of mechanoperception**—Most studies designed to investigate the growth response associated with mechanoperception have been conducted on actively growing plants. Very little is known regarding the ability of a plant to perceive a mechanical signal during a “nongrowing” or dormant period and to respond in the subsequent growing season. Valinger et al. (1994) provide the first evidence of a possible memory in plants to record mechanical loading during winter dormancy. They reported in their study on Pinus sylvestris L., that trees can perceive a periodic bending stress when kept at −6°C. When the trees were moved to conditions favorable for growth, the bent trees had a thigmomorphogenic response when compared to nonbent control trees. Past studies on dormancy and seasonal mitotic activity in apical...
buds (Carlson et al., 1980; Carlson, 1985) and more recent genomic studies (see for example Ko et al., 2006) have shown that even during so-called periods of dormancy, plants maintain an active level of metabolism. However, the nature of the stored mechanical message and how it stimulates growth after dormancy is still unknown.

**Physiological responses to mechanoperception**—A generalized flow chart of physiological responses to thigmomechanoperception is presented in Fig. 1. The first detectable response to a mechanical signal is a change in action potentials and electrical resistance, which occurs within seconds after perturbation (Sibaoka, 1966; Pickard, 1971; Jaffe, 1976), while mechanical shaking of stems blocks phloem transport within 1 to 2 min after perturbation (Jaffe and Telewski, 1984; Jaeger et al., 1988). The next measurable change occurs as an increase in intracellular Ca\(^{2+}\) (for review, see Knight, 2000). For this reason, it is currently unclear if stretch-activated Ca\(^{2+}\) channels (Ding and Pickard, 1993; Pickard and Fujiki, 2005) function in the primary perception or are triggered by the mechanosensing network proposed by Baluska et al. (2003). The function of stretch-activated channels is to facilitate the transport of Ca\(^{2+}\) across the cell membrane and into the cell in response to mechanical stress (Ding and Pickard, 1993; Pickard and Fujiki, 2005). An increase in cytoplasmic calcium in response to mechanical stress has been documented in several plant systems (Toriyama and Jaffe, 1972; Knight et al., 1991, 1992; Trewavas and Knight, 1994; Legué et al., 1997; Pickard and Fujiki, 2005).

Hydrogen peroxide (H\(_2\)O\(_2\)) and other reactive oxygen species (ROS) are part of the defense response of plants, for example, in fungal attack involving mechanical insertion via growth of a fungal penetration peg through the host cell wall (for review, see Sutherland, 1991; Mehdy, 1994). Yahraus et al. (1995) induced an oxidative burst in cultured soybean [Glycine max (L.) Merrill] cells in response to osmotic stress (altered turgor pressure) and direct physical pressure (mechanical stress). The induction of ROS and an increase in cytosolic Ca\(^{2+}\) appear to be concurrent, and ROS have been suggested to regulate Ca\(^{2+}\) channel gating (Mori and Schroeder, 2004).

Jaffe (1976) reported mechanical stress caused a complete cessation of elongation growth 6 min after force application, with the growth rate resuming after 15 to 30 min in Phaseolus vulgaris. Coutand et al. (2000) reported similar results with a cessation of elongation growth 8.3 ± 3.7 min after induction of a mechanical stress in the basal stem of tomato plants; elongation ceased and subsequently required 60.3 ± 3.5 min of recovery time before a normal rate of elongation growth resumed. They concluded that this is evidence for a rapid, acropetally transmitted signal from the point of flexure to the actively elongating zone directly below the apical meristem. The existence of an acropetally transmitted thigmomorphogenetic signal was first reported by Emmer et al. (1980), who also concluded the transportable factor was not ethylene. Subsequently, Takahashi and Jaffe (1984) reported the presence of phytoalexin-like substances in extracts of mechanically perturbed plants, which peaked in concentration 1 h after a application of a mechanical stress and, when applied to nonstressed plants, elicited a thigmomorphogenetic-like response.

Apparent cessation of elongation growth may actually precede the rapid up regulaton (expression) of calmodulin and calmodulin-related genes (TCH1, TCH2, TCH3), which was observed in Arabidopsis 10 to 30 min after mechanoperception of a touch, wind, or rain stimulus and returned to base levels by 1 to 2 h (Braam and Davis, 1990). Arabidopsis TCH3 encodes for a Ca\(^{2+}\) binding protein which is expressed in response to both externally applied mechanical forces and internally generated growth strains during tissue development in the absence of an external mechanical stress (Sistrunk et al., 1994). Arabidopsis TCH4 encodes for a xyllo glucan endotransglycosylase and was co-expressed with the other TCH genes (Xu et al., 1995). The expression of xyllo glucan endotransglycosylase in response to wind was located in cells undergoing expansion (Antosiewicz et al., 1997).

Touch, wind, and wounding all induced increased lipoxygenase (LOX) mRNA transcription in wheat (Triticum aestivum L.) seedlings (Mauch et al., 1997). The mechanical stress induced response occurred within 1 h after treatment, and the amount of transcript was reported to be strongly dose-dependent. LOXs are involved or implicated in a number of metabolic pathways associated with plant growth and development, ABA biosynthesis, senescence, mobilization of lipid reserves, wound responses, resistance to pathogens, formation of fatty acid hydroperoxides, and synthesis of jasmonic acid and traumatic acid (for review, see Mauch et al., 1997).

---

**Fig. 1.** Flow chart of the time course of physiological and growth response to mechanical stress. See text for citation information.
As previously mentioned, callose synthesis and deposition are induced by flexing mechanical stress in the phloem of bean (*Phaseolus vulgaris*) and loblolly pine (*Pinus taeda*) stems 1 h after mechanical stimulation, peaking after 9 h and being re-absorbed by 25 h (Jaffe and Telewski, 1984; Jaffe et al., 1985). Callose deposition also occurs within 5 min of gravity stimulation in *Zea mays* and *Pisum sativum*. Deposition of callose occurred first on the upper side of displaced stems, and after 2–3 h, the pattern was reversed. The callose inhibitor, 2-deoxy-D-glucose (DDG), blocked callose formation and considerably reduced gravitropic bending in both species (Jaffe and Leopold, 1984). Sound in the ultrasonic range was reported to induce transient callose formation in cotton seed (Currier and Webster, 1964).

Ethylene biosynthesis has been reported to be a fairly ubiquitous response to a number of environmental stresses, including mechanical stresses (for review, see Abeles et al., 1992; Bleecker and Kende, 2000), and several researchers have suggested ethylene serves as a signaling molecule. Wind, touch, and dynamic flexing have all been shown to induce ethylene formation in vascular plants (for review, see Telewski, 1995), and ethylene has been reported to be involved in the gravitropic response, a response to displacement resulting in static bending with respect to the gravitational force vector (Savidge et al., 1983; for review, also see Steed et al., 2004). Ethylene production in response to mechanical stress peaks 2 h in *Phaseolus vulgaris* (Biro and Jaffe, 1984), and 9 h in *Pinus taeda* (Telewski and Jaffe, 1986) after force application. Increased cell divisions by the vascular cambium occurred within 6 h after the application of mechanical stress in *P. vulgaris* (Biro et al., 1980). The role of ethylene in response to mechanical stresses appears to affect secondary growth and subsequent development and differentiation of the vascular cambium and not impact primary growth (elongation or height growth) associated with apical meristems (Coutand et al., 2000; for review, see Braam, 2005).

The role of auxin in gravitropism has been studied for almost 80 years, and its role in plant tropisms leading to the postulation of the Cholody–Went hypothesis (Went and Thimann, 1937) has stood the test of time (Gutjahr et al., 2005; Esmon et al., 2006). Surprisingly, little information exists on the role of auxin and other plant growth regulators in the thigmomorphogenetic response. Ermer and Jaffe (1982) reported the accumulation of auxin-like substances and higher levels of abscisic acid (ABA) in response to mechanical bending. These authors hypothesized the accumulation of these plant growth regulators resulted from ethylene production earlier in the thigmomorphogenetic response and was responsible for the reduction in internode (shoot) elongation. However, Johnson et al., (1998) challenged this hypothesis when they observed ethylene mutants still respond to mechanical perturbations with a reduction in shoot elongation. The role of plant growth regulators in the post mechanoperception-thigmomorphogenetic response is still wide open for investigation.

**Future areas for investigation**—As is evidenced in this review, the field of study on mechanosensing and mechanoperception in plants is progressing rapidly and supports the proposal of a unified hypothesis of plant perception of the mechanical environment. One area stands out, which requires further elucidation, specifically integrating the role of the sensing network at the cytoskeleton–plasma membrane–cell wall linkage with the plasmodesmata (Baluška et al., 2003, 2005) and the presence of mechanosensory calcium-selective cation channels (Cosgrove and Hedrich, 1991; Ding and Pickard, 1993; Dutta and Robinson, 2004) and the plasmalemmal reticulum (Gens et al., 2000; Pickard and Fujiki, 2005).

Are they competing models for a mechanosensory systems in plants, or are they interconnected and co-functional, and what is the specific signal that is transmitted by the network?

Another area of interest focuses on how plants differentiate between the various mechanical signals. At the most simplistic level, from mechanoperception of a mechanical signal by a plant cell to the cascade of initial physiological responses, there appears to be very little difference in the response pathway that would allow for discrimination in programmed reaction to the variety of mechanical stresses present in the environment. An example is the differentiation of response between gravitropism vs. thigmomorphogenesis in woody plants. Reaction wood formation is a well-documented gravitropic response in woody plants (for review, see Timell, 1986a–c). A multitude of field observations suggest that wind can stimulate the vascular cambium to produce reaction wood in the tree trunk, even if the tree shows no signs of having been displaced with respect to gravity (see Timell, 1986c). Experimentally, Larson (1965) also reported the formation of compression wood in *Larix* seedlings exposed to an artificial unilateral wind. Although it appears that mechanoperception in plants can be very rapid, the threshold of exposure or presentation to a gravitropic stimulus may require several minutes to hours (the presentation time) (for review, see Timell, 1986c). If a plant is displaced with respect to gravity and returns to its vertical orientation before the presentation time is met, the plant will not exhibit gravitropic curvature. In order to isolate a gravitropic response from a thigmomorphogenetic response, Telewski (1989) carefully flexed stems of *Abies fraseri* (Pursh.) Poir. and returned them to the vertical orientation before the required presentation time for initiation of a gravitropic response. The wood produced in response to this dynamic flexure possessed characteristics that were intermediate between normal wood and compression wood. It would be interesting and useful to sort out how the mechanosensory system of plant cells discerns and differentiates between the various mechanical forces imposed upon the plant. What is the timing mechanism required in meeting the presentation time of the gravitropic response in response to static displacement and how does this differ from the perception of dynamic swaying? For example, how does the mechanosensory system distinguish between gravity, wind sway, vibration, and sound?

The application of current state-of-the-art analytical tools, such as in situ hybridization and visualization methods for locating gene expression and plant growth regulators, would provide more detailed information on the putative roles of auxins and other plant growth regulators to further characterize the thigmomorphogenetic response pathway. Finally, as mentioned by Braam (2005), the application of molecular biological methods, including microarrays and bioinformatics, will further elucidate the array of plant responses to mechanical stresses and may help to identify the different physiological responses that occur after mechanoperception and to define developmental changes specific to a specific mechanical signal.

**LITERATURE CITED**


Lintilhac, P. M., and T. B. Vesecky. 1981. Mechanical-stress and cell-wall orientation in plants. 2. The application of controlled directional


Lloyd, F. E. 1942. The carnivorous plants. *Chronica Botanica* Waltham, Massachusetts, USA.


