14 Electrophysiology in Mechanosensing and Wounding Response

TERUO SHIMMEN

14.1 Mechanosensing

14.1.1 Responses of plants to mechanical stimulus

Touch induces rapid leaf movement in "sensitive" plants such as *Mimosa pudica*. This characteristic response indicates the recognition of mechanical stimuli, and it is termed thigmonasty or seismonasty. On the other hand, "insensitive" plants move passively in response to touch. The passive movement is believed to indicate a lack of recognition. However, this is not the case. After daily touching, the plants become shorter and thicker. Increased thickness reinforces the physical strength of the plant, therefore reducing the impact of mechanical stress on the plants. According to Jaffe and Forbes (1993), this phenomenon is termed thigmomophogenesis. Aequorin-expressing, transgenic plants undergo increases in cytoplasmic free Ca^{2+}, a second messenger (Knight et al. 1992). Thus, it is clear that even insensitive plants can sense mechanical stimuli.

In the plasma membrane, ionic processes are believed to play important roles in stimulus-perception and signal processing. Electrophysiological techniques are useful when monitoring the rapid ionic processes in the mechanosensing of plants.

14.1.2 Receptor potential in higher plants

Since the perception of the stimuli can easily be visualized, action plants are frequently utilized in mechanosensing analysis. *M. pudica* is the most common plant used in this type of investigation (Fig. 14.1). The leaves are equipped with three types of motile structures: main pulvinus (primary pulvinus), sub-pulvinus (secondary pulvinus) and pulvinule (tertiary pulvinus). These structures are responsible for movement of the petiole, pinna, and leaflet, respectively. The mechanosensitive leaf movement of *M. pudica* has been extensively studied. In the first step of mechanoperception, mechanical

Department of Life Science, Graduate School of Life Science, Himeji Institute of Technology, Harima Science Park City, Hyogo, 678-1297 Japan

Plant Electrophysiology – Theory & Methods (ed. by Volkov)

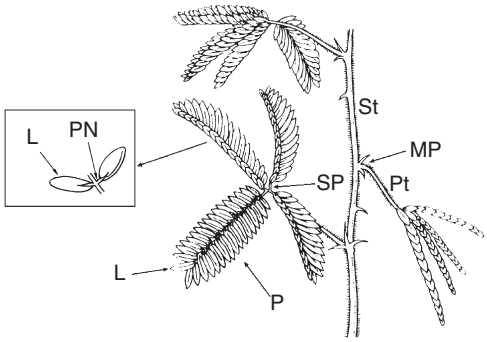

Fig. 14.1. Leaf movement of *Mimosa pudia*. Leaf is equipped with three kinds of motile structures, main pulvinus (*MP*), sub-pulvinus (*SP*) and pulvinule (*PN*), and responsible for movement of petiole (*Pt*), pinna (*P*) and leaflet (*L*), respectively. *St* stem. *Left leaves*: before stimulation. *Right leaf*: after stimulation. (Modified from Ishikawa 2003)

stimuli must be transformed into electrical signals or receptor potentials. Sibaoka (personal communication) suggested that pulvini are mechanosensitive organs. However, the sensory cell has not been identified in this material.

Receptor cells have been identified in action plants such as the terrestrial *Dionaea muscipula* and aquatic *Aldrovanda vesiculosa*. Although their habitats are different, both plants are carnivorous. The leaves are modified as traps for prey, and each trap is composed of paired lobes containing sensory hairs. The lobes are connected by the motile midrib (Fig. 14.2). When the sensory hairs are bent by the prey, the trap quickly closes to catch the prey.

In case of *A. vesiculosa*, each lobe has 20 sensory hairs. Individual hairs have four small sensory cells. Using sophisticated techniques, Iijima and Sibaoka analyzed the receptor potentials and action potentials (Sibaoka 1991) (Fig. 14.3). One microelectrode was inserted into a sensory cell and other into an epidermal cell of a lobe. When a stimulus was applied by bending the hair, the receptor cell showed depolarization. The amplitude of the depolarization increased as the bending force increased, indicating that the response is a receptor potential. When depolarization reaches its threshold level, an action potential is recorded in a cell of a lobe. This action potential is transmitted to the midrib of the trap, inducing closure. The receptor potentials and action potentials were generated upon mechanical stimulation. Voltage-sensitive and mechanosensitive ion channels should be involved, respectively.

14.1.3 Analysis of receptor potential in Characean cells

Internodal cells of Characeae have been useful materials for the study of plant electrophysiology for the following reasons: (1) due to the simple morphology, the electrical responses of a target cell can be easily measured, (2) since

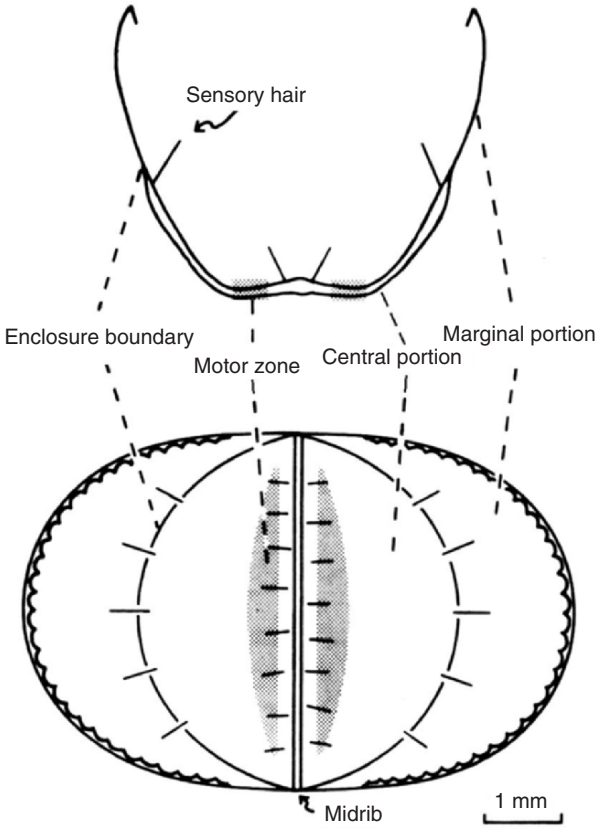

Fig. 14.2. Trap of *Aldrovanda vesiculosa*. Cross-section (*upper*) and upper view (*lower*). (Modified from IIjima and Sibaoka 1981)

internodal cells are large and cylindrical, various cell surgery techniques such as cutting, ligating and intracellular perfusion, can be applied, and (3) Since Characeae is an aquatic plant, electrical measurement can be carried out under their native external condition, aquatic solution. Three genera of Characeae are generally used: *Chara, Nitella* and *Nitellopsis*.

Kishimoto (1968) was the first to successfully record a receptor potential upon mechanical stimulation in Characeae. He stimulated one portion of an internodal cell using an electromagnet. Staves and Wayne (1993) stimulated an internodal cell of *Chara* using a micromanipulator analyzing action potentials but not receptor potentials. Shimmen (1996) developed a simple method to analyze receptor potentials and action potentials induced by mechanical stimulation (Fig. 14.4). A cell is separated into two halves, and the potential difference between pools A and B is measured. A small glass rod (stimulator) is placed on the cell. The cell is stimulated by dropping glass tubing onto the

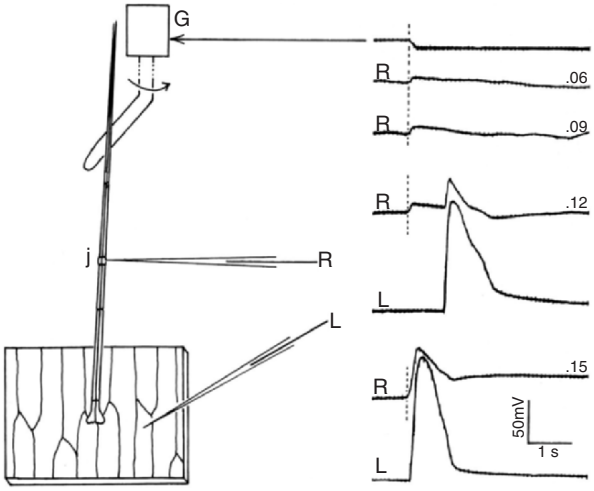

Fig. 14.3. Measurement of electrical responses in *Aldrovanda vesiculosa* on mechanical stimulation. **A** Microelectrodes were inserted into either a sensory cell at the joint (*j*) of a sensory hair (*R*) and into a epidermal cell of lobe (*L*). *G* Distal position of the hair is pushed with a fine glass rod connected to a pen-writing galvanometer. **B** Electrical responses in sensory cell (*R*) and lobe cell (*L*). (Modified from Sibaoka 1991)

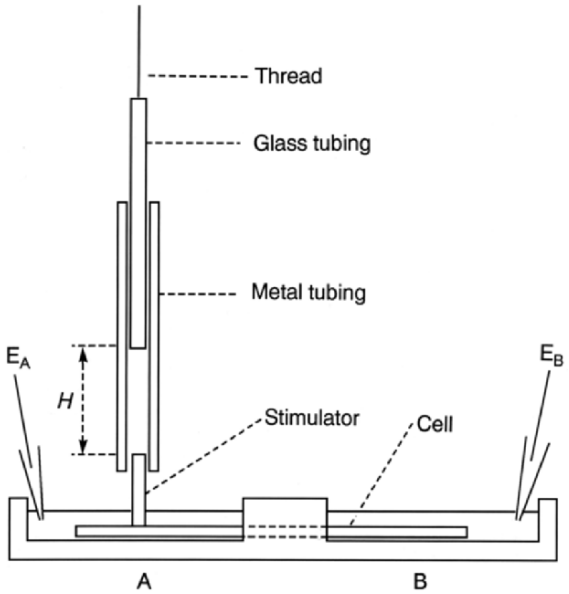

Fig. 14.4. Apparatus for measurement of receptor potential and action potential in internodal cell of *Characeae* upon mechanical stimulation. An internodal cell is separated into two halves (**A**, **B**) and the potential difference is measured using two electrodes (E_A and E_B). *H* height from which glass tubing is dropped. Fixed metal tubing is used as a guide to drop glass tubing onto Stimulator. (Cited from Shimmen 1996)

stimulator. The strength of the stimulation can be controlled by either changing the weight of the glass tubing or changing the height from which the glass tubing is dropped.

A typical measurement is shown in Fig. 14.5. The strength of stimulation was controlled by changing the height from which the glass tubing was dropped. With an increase in stimulation strength, the amplitude of the receptor potential also increased. When depolarization reached the threshold level, an action potential was induced in the cell part in pool A due to activation of a voltage-sensitive channel. It transmitted to the cell part in pool B.

To analyze the ionic mechanism for generation of receptor potentials, the membrane potential must be measured but not the change in membrane potential, since the relation between membrane potential and equilibrium potential for ions across the membrane must be compared. It is not recommended to use microelectrodes in studies where drastic mechanical stimulation is applied to the cell. In Characean cells, membrane potential can be measured without inserting microelectrodes (Shimmen et al. 1976). When the external K^+ concentration was increased up to 100 mM in pool B (Fig. 14.4),

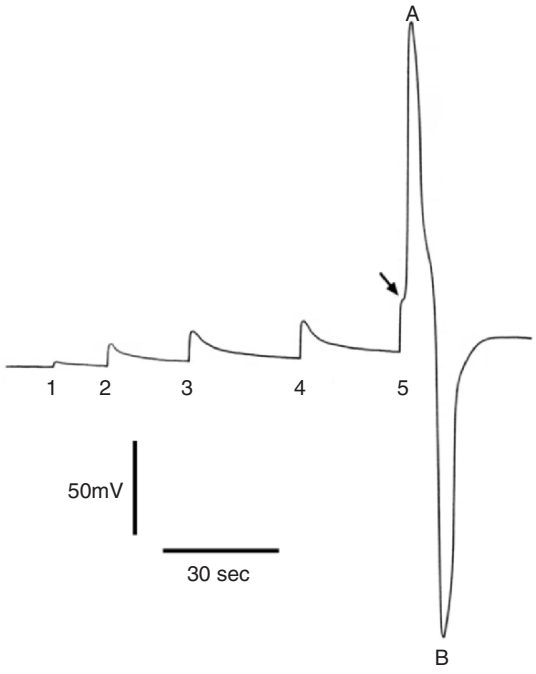

Fig. 14.5. Receptor potentials and action potentials induced by mechanical stimulation in *Chara* internodal cell. Cell was stimulated using a apparatus shown in Fig. 14.4. Numbers in the figure show height from which a glass tubing (1.3 g) was dropped. When depolarization of a receptor potential reached a threshold (*arrow*), action potential was induced first in the cell part in pool A (**A**) and then it was transmitted to the cell part in pool B (**B**). (Cited from Shimmen 1996)

membrane potential of the cell part B depolarizes to about 0 mV. Therefore, potential difference measured between pools A and B represents membrane potential of the cell part in the pool A. Sorbitol (180 mM) isotonic to 100 mM KCl must be added to pool B.

Membrane potentials of plant cell comprise two components: a passive diffusion component and an active component generated by electrogenic H^+-pump (Shimmen and Tazawa 1977). The resting membrane potential of *Chara* is more negative than –200 mV. Two possibilities are suggested for membrane depolarization upon mechanical stimulation: either inhibition of the electrogenic proton pump or change of ion channel permeability. When cells were treated with N, N′-cyclohexylcarbodiimide, an inhibitor of H^+-pump, the resting membrane potential depolarized to about –130 mV indicating that the pump was inhibited. However, the amplitude of receptor potential did not change after inhibition of the pump (Shimmen 1997a). In addition, it was found that electrical resistance decreased during generation of a receptor potential (Shimmen 1997c). These results indicate that a receptor potential is generated by activation of ion channel(s) but not by inhibition of the electrogenic H^+-pump.

Ca^{2+} and Cl^- channels are candidates for membrane depolarization in the generation of receptor potentials since the equilibrium potential of these ions across the plasma membrane are positive inside. Various inhibitors of these ion channels failed to inhibit receptor potentials (Shimmen 1997a). However, more recent experiments indicated that activation of the Cl^- channel is involved (Fig. 14.6). When 100 mM KCl was added to the external medium, membrane potential depolarized to 0 mV. The membrane potential changed

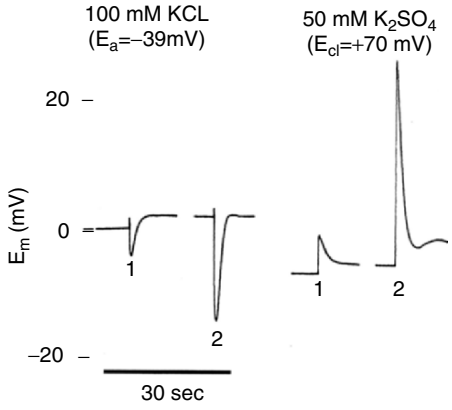

Fig. 14.6. Electric response induced by mechanical stimulation of *Chara* cell under K^+-induced depolarization. By applying 100 mM K^+ to the external medium, membrane potential depolarize to the level close to 0 mV. Upon mechanical stimulation, response to the negative direction is induced in the presence of 100 mM KCl and that to the positive direction in the presence of 50 mM K_2SO_4 (1.3 mM Cl^-). Numbers in the figure show the height from which glass tubing was dropped. Equilibrium potential for Cl^- across the plasma membrane (ECl^-) is shown. (Modified from Shimmen 1997b)

to the negative direction upon mechanical stimulation. When 50 mM K_2SO_4 (containing 1.3 mM Cl^-) was added to the external medium, the membrane potential changed to the positive direction upon mechanical stimulation. Assuming the Cl^- concentration of the cytopolasm to be 21 mM (Tazawa et al. 1974), equilibrium potential for Cl^- is calculated to be –39 mV in the presence of 100 mM Cl^- and +70 mV in the presence of 1.3 mM Cl^- in the external medium. Thus, the membrane potential changes to the direction of equilibrium potential for Cl^- upon mechanical stimulation, indicating activation of the Cl^- channel (Shimmen 1997b).

Kaneko et al. (2005) found an increase in cytoplasmic Ca^{2+} upon mechanical stimulation. They microinjected aequorin into the cytoplasm. Since an action potential was generated by activation of both Ca^{2+} and Cl^- channels (Shimmen et al. 1994), cytoplasmic Ca^{2+} also increased (Williamson and Ashley 1982). Therefore, experiments were carried out in the presence of a high K^+ concentration which inhibits generation of an action potential. Mechanical stimulation induced a significant increase in light emission from cytoplasmic aequorin, suggesting that Ca^{2+} channel was activated. One of the characteristics of the receptor potential is its graded increase to stimulus intensity. Since the extent of light emission was dependent on the amplitude of stimulation, it was suggested that the Ca^{2+} channel be dedicated to the generation of receptor potentials. To answer the question of which channel is mechanosensitive or if both channels are mechanosensitive, it has been reported that the characean plasma membrane is equipped with the Ca^{2+}-activatable Cl^- channel (Shiina and Tazawa 1988; Mimura and Shimmen 1994). Therefore, a possibility is suggested that Cl^- channel is activated by Ca^{2+} flowing into the cell through mechanosensitive Ca^{2+} channels. However, the possibility still remains that both channels are mechanosensitive.

14.1.4 Stretch-activated channel

Development of the patch-clamp method brought revolutionary change in electrophysiology. This made it possible to analyze close-open response in a single ion channel. This method also made it possible to identify stretch-activated ion channels (SA channel). SA channels are activated by the stretching of the membranes due to application of suction force to a measuring pipette. Such SA channels have been identified in epidermal cells of higher plants. Ding and Pickard (1993) found Ca^{2+}-selective SA channels. Falk et al. (1988) and Qi et al. (2004) found anion-selective SA channels. However, it cannot be concluded that these SA-channels are involved in mechanosensing.

14.1.5 Signal transmission by action potential

The occurrence of action potentials has been reported in various plant materials (Davies 1987). As seen in *D. muscipula* and *A. vesiculosa*, an action potential is initiated by a receptor potential and transmitted to the motile

cells of midrib to induce movement. Thus, an action potential is dedicated to the transmission of signals in plants as in the case of animal nerve cells. In both carnivorous plant species, the peak potential of action potentials changes to the positive direction when extracellular Ca^{2+} concentration was increased (Iijima and Sibaoka 1985; Hodick and Sievers 1986), suggesting that the Ca^{2+} channel is dedicated to the generation of action potentials.

14.1.6 Ionic mechanism of action potential analyzed in Characeae

Ionic mechanism of action potentials has been studied extensively in characean cells. Since the generation of action potential is dependent on both intracellular and extracellular chemical composition, modification of chemical composition at both sides of the plasma membrane is recommended. The development of an intracellular perfusion technique made it possible to control intracellular chemical composition in characean cells (Tazawa et al. 1976). After cutting both ends of an internodal cell, vacuolar space is perfused with an artificial medium. By this procedure, natural vacuolar sap is replaced with a perfusion medium (Fig. 14.7b). When a Ca^{2+}-chelator is contained in the perfusion medium, the tonoplast is disintegrated (tonoplast-free cell) (c). By the following perfusion, chemical composition inside the plasma membrane can be thoroughly controlled (d). Using this tonoplast-free cell, ATP-dependent generation of the membrane potential due to the activity of the electrogenic H^+ pump was unequivocally demonstrated (Shimmen and Tazawa 1977).

Since both equilibrium potentials for Ca^{2+} and Cl^- are inside positive, involvement of these ions in generation of action potentials has been suggested: influx of Ca^{2+} and efflux of Cl^-. When cytoplasmic Cl^- concentration was decreased to 0.01 mM in tonoplast-free cells, action potentials were still generated (Shimmen and Tazawa 1978). Since the Cl—hypothesis was prevalent at that time, this result was confusing. However, this suggested that the action potential was generated by activation of Ca^{2+} channel. Accordingly, increase in Ca^{2+} influx upon generation of action potentials was demonstrated (Hayama et al. 1979; Shiina and Tazawa 1987b).

Upon membrane excitation, increase in Cl^- efflux had also been reported (Oda 1976; Kikuyama 1986a; Shiina and Tazawa 1987a), indicating activation of Cl^- channel upon excitation. Analysis using tonoplast-free cells demonstrated the presence of Ca^{2+}-activated Cl^- channel in the plasma membrane. When intracellular Ca^{2+} was increased by the perfusion, Cl^- efflux increased significantly (Shiina and Tazawa 1988; Mimura and Shimmen 1994). Thus, it is believed that Ca^{2+} flow into the cell activates the Ca^{2+}-activated Cl^- channel of the plasma membrane. In tonoplast-free cells, Cl^- efflux during membrane excitation is not observed (Kikuyama et al. 1984). This is reasonable since Ca^{2+} should be bound by a Ca^{2+} chelator,

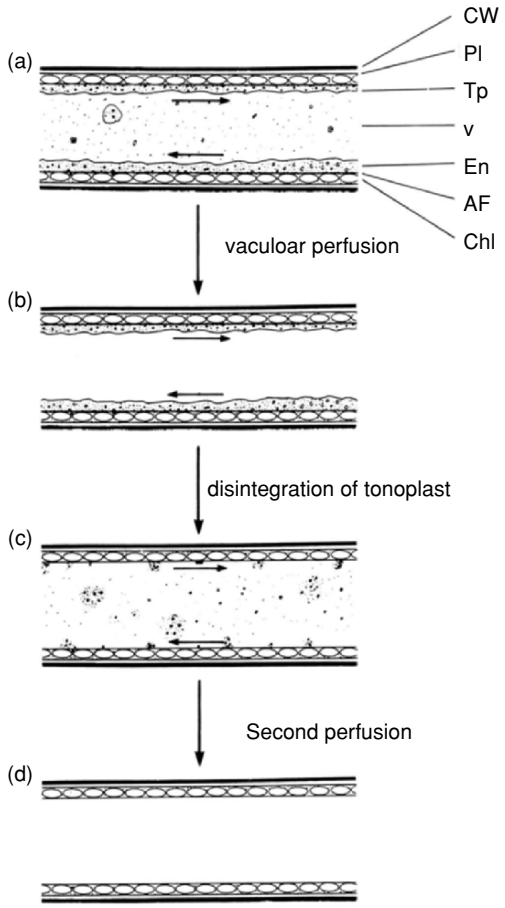

Fig. 14.7. Intracellular perfusion in internodal cells of Characeae. (a) Before treatment. (b) After butting both cell ends, the vacuolar sap was replaced with an artificial medium by intracellular perfusion. (c) Disintegration of tonoplast (tonoplast-free cell). Perfusion medium and cytoplasmic component is mixed. (d) By intracellular perfusion, chemical composition just inside the plasma membrane can be controlled. *CW* cell wall, *Pl* plasma membrane, *Tp* tonoplast (vacuolar membrane), *V* vacuole, *En* streaming sol endoplasm, *AF* bundles of actin filament responsible for cytoplasmic streaming, *Chl* chloroplast fixed to the gel layer. *Arrows* indicate direction of cytoplasmic streaming. (Cited from Shimmen 1988)

which had been introduced into the cell by the intracellular perfusion. It was found that significant increase in Cl^- efflux did not occur occasionally, even when an intact cell generated an action potential (Kikuyama et al. 1984). It is believed that activation of Cl^- channel was uncoupled because of unidentified mechanism. Thus, both Ca^{2+} and Cl^- channels are involved in generation of action potentials in Characeae.

14.1.7 Control by action potential

14.1.7.1 Turgor movement in M. pudica

Transmission of signals via action potentials in *D. muscipula* and *A. vesiculosa* is analogous to that of nerve cell. When an action potential of an axon reached the surface of the skeletal muscle, an action potential was generated at the plasma membrane of the muscle cell, triggering contraction. Thus, the action potential of the effector cell causes a specific response. A similar situation can be envisaged in *M. pudica*. When an action potential of the petiole reached a main pulvinus, an action potential is induced in cortex cells of the pulvinus, and downward movement of the petiole is induced (turgor movement). Samejima and Sibaoka (1980) inserted Cl—sensitive electrodes into the cortex of the main pulvinus. Since the tip of the electrode is located in the apoplast (cell wall outside of the cortex cell), an increase in Cl^- concentration indicates efflux of Cl^- from cortex cells. Action potentials are observed in both upper and lower sides of the pulvinus upon stimulation. However, significant efflux of Cl^- occurs only in cells of the lower side (Fig. 14.8). Kumon and Tsurumi (1984) found a significant increase in extracellular K^+ at the lower cortex of a main pulvinus in photo-stimulated movement. Thus, significant ion efflux occurs in the lower cortex but not in the upper cortex of the main pulvinus. The imbalance of turgor pressure between upper and lower parts causes quick downward movement of the petiole. Involvement of protein phosphorylation in this turgor movement was reported (Kameyama et al. 2000). The relation between protein phosphorylation and turgor movement remains to be solved.

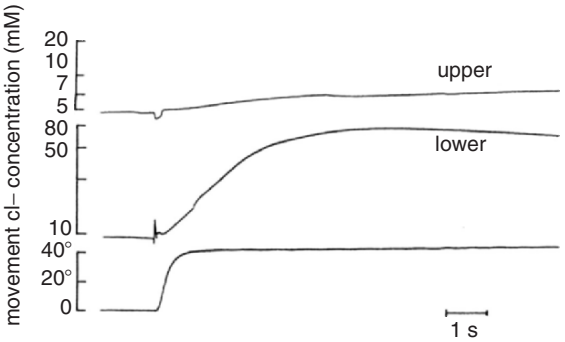

Fig. 14.8. Cl^- efflux from cortex cells of main pulvinus of *Mimosa pudica*. Cl^- electrodes had been inserted into the extracellular space of cortex. When cortex cells generated an action potential, Cl^- efflux (increase in extracellular Cl^- concentration) occurs in lower cortex but not in upper cortex. (Cited from Samejima and Sibaoka 1980)

14.1.7.2 Intracellular signal processing induced by action potential

In *M. pudica*, intracellular processes induced by action potential are not known. In Characeae, however, signal processing upon excitation of the plasma membrane has been studied extensively. It is believed that Ca^{2+} flows into the cell via voltage-sensitive Ca^{2+} channels during action potential. Increase in cytoplasmic free Ca^{2+} during membrane excitation has been demonstrated using aequorin emission (Williamson and Ashley 1982; Kikuyama and Tazawa 1983). Increased cytoplasmic Ca^{2+} works as a second messenger to induce physiological phenomena.

14.1.7.2.1 Tonoplast action potential

When an action potential was generated at the plasma membrane, a potential change at the tonoplast to the inside positive direction was induced (tonoplast action potential: references cited in Shimmen and Nishikawa 1988). In most characean species, it is difficult to distinguish between action potentials at the plasma membrane and the tonoplast. In *Nitella flexilis*, however, generation of these action potentials is temporally separated (Shimmen and Nishikawa 1988). In Fig. 14.9, an internodal cell of *N. flexilis* was mechanically stimulated. When the membrane potential reached its threshold upon generation of a receptor potential, quick action potential at the plasma membrane is induced (arrow) and then the slow action potential at the tonoplast is induced.

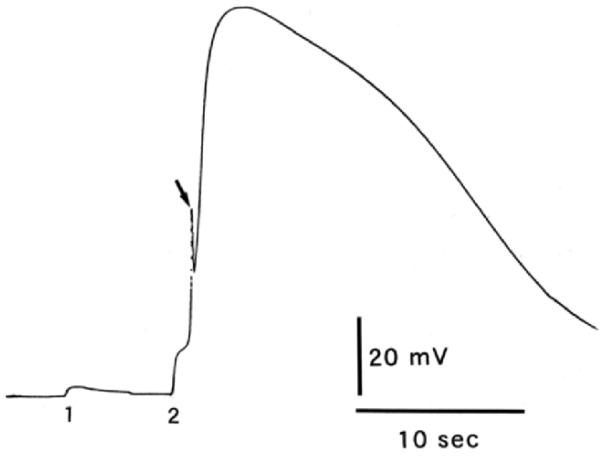

Fig. 14.9. Tonoplast action potential in *Nitella flexilis*. Internodal cell was mechanically stimulated as shown in Fig. 14.4. Numbers show height from which glass tubing was dropped. When glass tubing was dropped from the position of 2 cm high, large receptor potential was generated and then an action potential at plasma membrane was (*arrow*). With a slight delay, large action potential at the tonoplast was induced (original figure)

Tonoplast action potential is caused by activation of the Cl^- channel of the tonoplast (Kikuyama and Tazawa 1976; Shimmen and Nishikawa 1988). Permeabilization of the plasma membrane by plasmolysis method, developed for studying cytoplasmic streaming of characean cells, was also useful for the analysis of the tonoplast function (Shimmen and Tazawa 1983). Using a permeabilized cell, the presence of two proton pumps in the tonoplast was unequivocally demonstrated (Shimmen and MacRobbie 1987). Kikuyama (1988) found that application of Ca^{2+} to the external medium induced Cl^- efflux from the vacuole in permeabilized *Nitella* internodal cells. In addition, injection of Ca^{2+} into the cytoplasm of an intact *Nitella* cell induced a potential change at the tonoplast (Kikuyama 1986b). These facts indicate that the characean tonoplast is equipped with a Ca^{2+}-activated Cl^- channel.

14.1.7.2.2 Cytoplasmic streaming

Characeae has been also a suitable material for studies on cytoplasmic streaming (cf. Kamiya 1986). It has been established that the motive force is generated by the actin–myosin system (Shimmen and Yokota 2004). As early as in 1921, Lauterbach found that the cytoplasmic streaming of characean cell stops upon application of the mechanical stimuli (Kamiya 1959). It became evident that action potentials mediate mechanoperception and stoppage of cytoplasmic streaming. Involvement of Ca^{2+} as a second messenger was first suggested by an experiment of Barry (1968). *Nitella* cells generated action potentials in the medium containing $CaCl_2$, $MgCl_2$, $BaCl_2$ or $SrCl_2$, suggesting that the voltage-sensitive channel responsible for generation of an action potential is permeable to these divalent cations in the *Nitella* species. In the presence of either $MgCl_2$ or $BaCl_2$, uncoupling between excitation and stoppage of streaming is induced. However, increase in $CaCl_2$ concentration in these media containing either $MgCl_2$ or $BaCl_2$ relieved the cell from uncoupling, suggesting an essential role of Ca^{2+}. Increase in Ca^{2+} influx (Hayama et al. 1979; Shiina and Tazawa 1987b) and that in cytoplasmic free Ca^{2+} (Williamson and Ashley 1982; Kikuyama and Tazawa 1983) suggest that Ca^{2+} is involved in stoppage of cytoplasmic streaming. Using permeabilized cell, cytoplasmic streaming was found to be inhibited by Ca^{2+} of physiological concentrations, μM order (Tominaga et al. 1983). Although involvement of protein phosphorylation was suggested based on pharmacological analysis, the relation between the increase in Ca^{2+} and protein phosphorylation has not yet been found (Tominaga et al. 1987).

14.1.7.2.3 Signal processing in characean cell upon mechanical stimulation

Figure 14.10 depicts the possible signal processing induced by action potential in characean cells. Upon mechanical stimulation, a receptor potential is generated due to the activation of mechanosensitive Ca^{2+} and/or Cl^- channel (not shown). When the membrane potential reached a threshold level, action potentials are generated due to the activation of voltage-sensitive Ca^{2+}

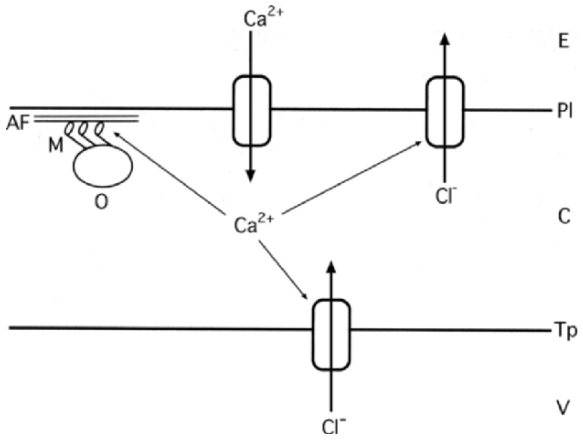

Fig. 14.10. Signal processing upon activation of voltage sensitive Ca^{2+} channel in characean cells. Ca^{2+} activates Cl^- channels of plasma membrane and that of tonoplast, and inhibits actin–myosin system. *Thick arrows* show flux of ions and *thin ones* show effect of Ca^{2+} *AF* bundle of actin filaments, *M* myosin, *O* organelle (original figure)

channel, resulting in the increase of cytoplasmic free Ca^{2+}. Ca^{2+} works as a second messenger and activates Cl^- channels of the plasma membrane and the tonoplast. It inhibits the actin–myosin system, causing stoppage of cytoplasmic streaming. It is not evident whether mechanosensitive and voltage-sensitive Ca^{2+} channels of the plasma membrane are the same or not. The possibility also remains that Ca^{2+} is mobilized not only from the cell exterior but also from intracellular organelles.

14.2 Wounding response

14.2.1 Electrical response in wounded plants

Plants show various responses to wounding and membrane depolarization is the earliest response shown. In some plants, electrical response is induced at the limited region near the wounding (Mertz and Hignbotham 1976; Stahlberg and Cosgrove 1994; Meyer and Weisenseel 1997). On the other hand, action potentials and/or variation potentials propagate systemically in many plants (Frachisse et al. 1985; Robin 1985; Robin and Bonnemain 1985; Julien et al. 1991; Julien and Frachisse 1992; Fromm and Eschrich 1993; Rhodes et al. 1996; Stankovic and Davies 1996). The shape and amplitude of variation potentials (or slow waves) are variable, showing a clear contrast with action potentials. Involvement of electrical signaling in systemic synthesis of proteinase inhibitor in wounded tomato plants clearly showed

the importance of membrane phenomena in the wounding response of plants (Wildon et al. 1992).

14.2.1.1 Leaf movement in Mimosa pudica

Signal transmission upon wounding had been extensively documented in *M. pudica*. Three types of signal has been suggested: m-wave (1.5–4 cm/s), s-wave (0.2–0.5 cm/s) and r-wave (6–12 cm/s). The entity of the m-wave is an action potential, and it cannot pass through the pulvinus. The s-wave is a variation potential associated with movement of unidentified substance. The s-wave can pass through a pulvinus. The entity of the r-wave has not been identified. Severe stimuli such as burning or cutting induce both the m-wave and the s-wave. Mild stimulation such as an electrical current or the application of cold water induces only the m-wave (Robin 1979; Sibaoka 1981).

When a severe stimulus was applied to one terminal leaflet of a pinna by heating, both the m-wave and the s-wave were generated [Fig. 14.11 (1)]. The action potential stimulated the pulvinule but could not pass through it. The s-wave entered the pinna-rachis, and an action potential was generated. This new action potential was transmitted basipetally and successively stimulated pulvinules to induce closure of the leaflets (2). The action potential stimulates the sub-pulvinus (1) to induce movement of the pinna. At this step, movement in the leaf was not observed for a while since the action potential could not pass the sub-pulvinus. During this quiet period, the s-wave moved basipetally in the pinna-rachis and entered the petiole. A new action potential was generated,

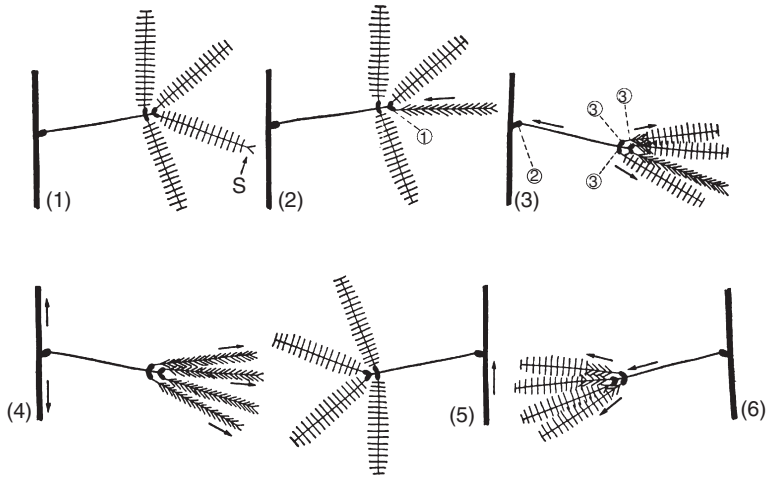

Fig. 14.11. Signal transmission upon wounding terminal leaflet as speculated from leaf movement. *s* Terminal leaflet was stimulating by heating. *Arrows* indicate the direction of signal transmission. For explanation, see text. (Cited from Sibaoka 1964)

and it stimulated three other sub-pulvini (3). It moved basipetally in the petiole and stimulated the main pulvinus (2).

The s-wave entered the pinna-rachis of the three other pinnas and generated new action potentials. These action potentials were transmitted acropetally and induced closure of leaflets in each pinna (3, 4) When the heat stimulus was strong enough, the s-wave entered the stem by passing through the main pulvinus and induced the movement in other upper and lower leaves (4–6). Thus, signal transmission can be observed without electrical instrument in *M. pudica*.

14.2.1.2 Propagation of action potential and variation potential

The generation of an electrical response in a leaf of *M. pudica* is shown in Fig. 14.12 (left). When an electric pulse was applied to the petiole, an action potential was transmitted basipetally. During the refractory period, burning of the terminal leaflet induced a variation potential, and it was transmitted to the petiole. The result in a tomato leaf is also shown in Fig. 14.12 (right). By burning of a leaflet blade, a variation potential was generated, and the petiole was transmitted basipetally. In both *Mimosa* and tomato, a spike was observed at the beginning of the variation potential. This nature has remained unsolved.

14.2.2 Analysis in Characean cells

When a plant suffered wounding, cells close to the dead cell were the first to perceive the "death signal". In higher plants, however, it is hard to pick up a

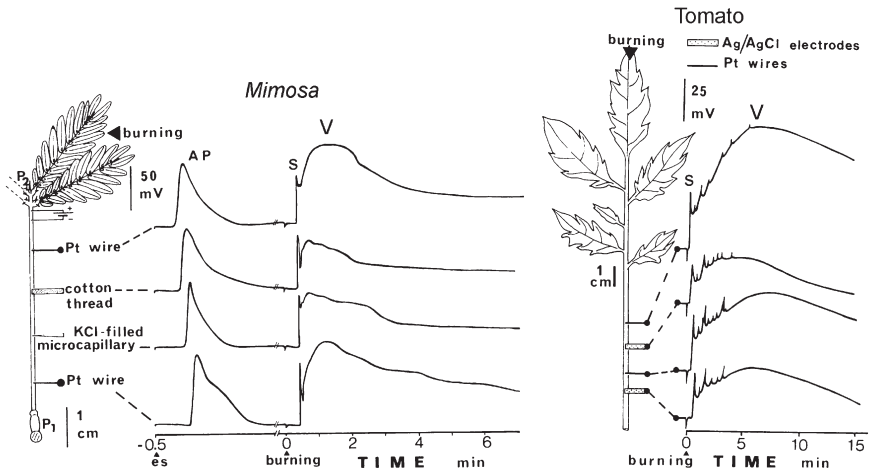

Fig. 14.12. Variation potential in *Mimosa* and tomato. *AP* action potential, *es* electrical stimulation, *s* spike, *V* variation potential. (Cited from Robin 1985)

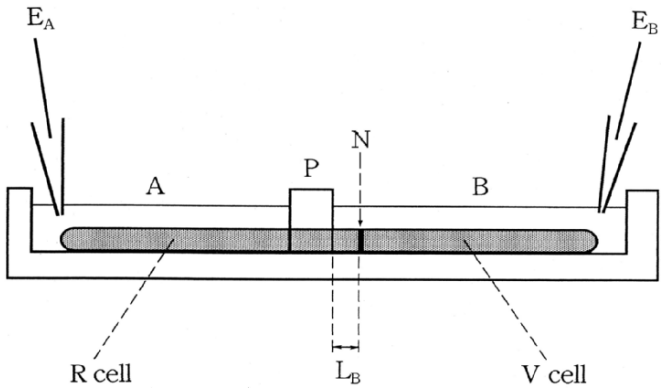

Fig. 14.13. Side view of experimental set up for analysis of wounding electrical response in Characeae. *R cell* receptor cell, *V cell* victim cell, *N* node, *A* and *B* pools, *P* partition between pools, L_B length of part of R cell in pool B (2 mm). E_A and E_B electrodes. (Cited from Shimmen 2002)

electrical signal from a target cell. Characeae can offer a suitable system for such analysis (Fig. 14.13). A specimen comprising two adjoining internodal cells is prepared. Two internodal cells are connected at the node. A specimen is mounted in the chamber so that one receptor cell (R cell) is situated in the 1 mm groove in the partition between two pools, A and B. The R cell is sealed into the groove at the partition with white Vaseline. The potential difference between the two pools is measured with electrodes (E_A and E_B). The length of the part of R cell in the pool B (L_B) is about 2 mm.

An example of measurement is shown in Fig. 14.14. When the victim cell (V cell) was cut, the membrane depolarized (a), and a long lasting component

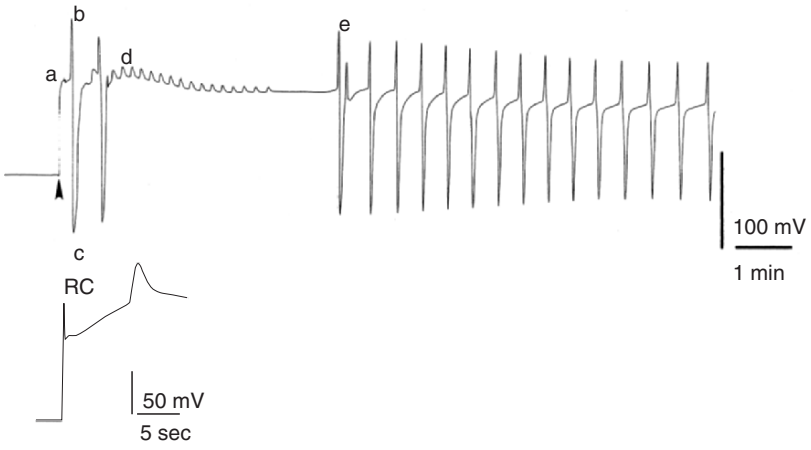

Fig. 14.14. Depolarization in wounding response of Characeae. At the time shown with an *arrow head*, V cell was cut (*upper record*). *Lower record* shows initial part of the response showing a rapid component (RC). For further explanation, see text. (Cited from Shimmen 2002)

continued for more than 30 min. During long-lasting depolarization, two components are superimposed. An action potential is generated in the cell part of R cell in the pool B (b), and it is transmitted to the cell part in the pool A (c). Then, small spikes are generated (d). Again, action potentials are generated (e). Just after cutting, a very rapid component is generated before the start of long-lasting component (Fig. 14.14 lower). When the amplitude of the long-lasting depolarization was large, this rapid component was masked (not shown). Systematic analyses showed that the long-lasting component and rapid components were generated at the end of the R cell in the pool B and that action potential is generated at the flank of the R cell. Rapid components and long-lasting depolarization are not transmitted. On the other hand, an action potential is transmitted in the longitudinal direction of an internodal cell. It is also transmitted to the neighboring internodal cell (Sibaoka and Tabata 1981). Thus, a long-lasting component is a type of receptor potential in wounding response, and this depolarization stimulates voltage-activated ion channels resulting in the action potential responsible for transmission of signals.

It is believed that K^+ released from the V cell is responsible for inducing the long-lasting depolarization (Shimmen, 2005, 2006). The amplitude of a long-lasting component decreases when the cell turgor pressure is decreased, indicating the stretching of the plasma membrane at the end of the R cell by the turgor pressure (Shimmen 2001). Thus, K^+-induced depolarization and membrane-stretching may be responsible for the generation of long-lasting component. When the electrogenic proton pump is inhibited by treatment with an inhibitor, the resting membrane potential is drastically depolarized. However, long-lasting component is generated, indicating that electrogenic proton pump does not play a central role (Shimmen 2001). The electrical resistance decreases during the depolarization (Shimmen 2005), indicating that the activation of ion channel(s) is involved. Pharmacological analysis failed to identify the ion channel involved. However, analysis under K^+-induced depolarization (c.f. Fig. 14.6) indicated the activation of Cl^- channel (Shimmen 2002). Thus, the electric phenomena in cells neighboring the dead cell can be analyzed by taking advantage of this material.

14.2.3 Transformation of pressure signal into electrical signal

In higher plants, it has been suggested that wound-induced hydraulic signals are the trigger for generation of variation potentials, i.e. transformation of pressure signal into electrical signal (Malone and Stankovic 1991; Malone 1992; Stankovic et al. 1997). The characean cell can be also a suitable material for the analysis of this phenomenon (Shimmen 2003). A specimen composed of a single internodal cell is prepared (Fig. 14.15, upper). One native end of the cell is removed by ligation and cutting. This cell now has a native nodal end and a ligated end. The cell is mounted so that the native node is located in pool B and the ligated end is located in pool A. The length of the

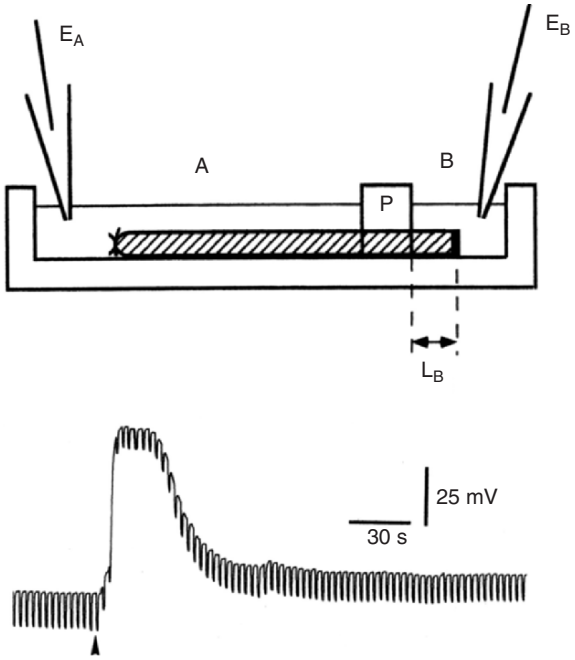

Fig. 14.15. Transformation of pressure signal into electrical signal in internodal cell of *Chara*. *Upper figure* shows apparatus and lower figure shows a record. Electric current pulses were applied between two pools during the measurement. *A* and *B* pools, *P* partition between pools, L_B length of cell part in pool B (about 2 mm), E_A and E_B electrodes. At the peak of depolarization, membrane resistance still seems high. However, most part of this resistance originates from resistance of intracellular fluid at the partition. (Cited from Shimmen 2003)

cell part in pool B is about 2 mm. When the osmolarity of pool B is increased, transient depolarization is generated (Fig. 14.15, lower). This response could also be induced when osmolarity of pool A was increased or if the osmolarity of both pools was increased. Thus, the decrease in cell turgor pressure is responsible and not the movement of water along osmotic pressure gradient. This response is generated at the distal end but not at the flank of the cell. Therefore, the response is not generated at the ligated end. In the measurement of Fig. 14.15 (lower), constant current pulses were applied, and the deviation of the membrane potential represents the change of the membrane resistance. Since it decreased during the depolarization, it is suggested that activation of ion channel is involved. The ion channel involved is remained to be identified. It is expected that this experimental set-up will open the way for the analysis of the transformation of a pressure signal to an electrical signal.

References

Barry WH (1968) Coupling of excitation and cessation of cyclosis in *Nitella*: role of divalent cations. J Cell Physiol 72:153–160

Davies E (1987) Action potentials as multifunctional signals in plants: a unifying hypothesis to explain apparently disparate wound responses. Plant Cell Environ 10:623–631

Ding JP, Pickard BG (1993) Mechanosensory calcium-selective cation channels in epidermal cells. Plant J 3:83–110

Falk LC, Edwards KL, Pickard BG, Misler S (1988) A stretch-activated anion channel in tobacco protoplast. FEBS Lett 237:141–144

Franchisse J-M, Desbiez O, Champagnat P, Thellier M (1985) Transmission of a traumatic signal via a wave of electric depolarization and induction of correlations between the cotyledonary buds in *Bidens pilosus*. Physiol Plant 64:48–52

Fromm J, Eschrich W (1993) Electric signals released from roots of willow (*Salix viminalis* L.) change transpiration and photosynthesis. J Plant Physiol 141:673–680

Hayama T, Shimmen T, Tazawa M (1979) Participation of Ca^{2+} in cessation of cytoplasmic streaming induced by membrane excitation in *Characeae* internodal cells. Protoplasma 99:305–321

Hodick D, Sievers A (1986) The influence of Ca^{2+} on the action potential in mesophyll cells of *Dionaea muscipula* Ellis. Protoplasma 133:83–84

Iijima T, Sibaoka T (1981) Action potential in the trap-lobes of *Aldrovanda vesiculosa*. Plant Cell Physiol 22:1595–1601

Iijima T, Sibaoka T (1985) Membrane potentials in excitable cells of *Aldrovanda vesiculosa* trap-lobes. Plant Cell Physiol 26:1–13

Jaffe MJ, Forbes S (1993) Thigmomorphogenesis: the effect of mechanical perturbation on plants. Plant Growth Regul 12:313–324

Julien JL, Frachisse JM (1992) Involvement of the proton pump and proton conductance change in the wave of depolarization induced by wounding in *Bidens pilosa*. Can J Bot 70:1451–1458

Julien JL, Desbiez MO, de Jaegher M, Frachisse JM (1991) Characteristics of the wave of depolarization induced by wounding in *Bidens pilosa* L. J Exp Bot 42:131–137

Kameyama K, Kishi Y, Yoshimura M, Kanzawa N, Sameshima M, Tsuchiya T (2000) Tyrosine phosphorylation in plant bending. Nature 407:37

Kamiya N (1959) Protoplasmic streaming. Protoplasmatologia VIII/3/a. Springer, Vienna

Kamiya N (1986) Cytoplasamic streaming in giant algal cells: a historical survey of experimental approaches. Bot Mag Tokyo 99:441–467

KanekoT, Saito C, ShimmenT, Kikuyama, M. (2005) Possible involvement of mechanosensitive Ca^{2+} channels of plasma membrane in mechanoperception in *Chara*. Plant Cell Physiol. 46: 130–135

Kikuyama M (1986a) Ion fluxes during excitation of Characeae. Plant Cell Physiol 27:1213–1216

Kikuyama M (1986b) Tonoplast action potential of Characeae. Plant Cell Physiol 27:1461–1468

Kikuyama M (1988) Ca^{2+}-increases the Cl^- efflux of the permeabilized *Chara*. Plant Cell Physiol 29:105–108

Kikuyama M, Tazawa M (1976) Tonoplast action potential in *Nitella* in relation to vacuolar chloride concentration. J Membr Biol 29:95–110

Kikuyama M, Tazawa M (1983) Transient increase of intracellular Ca^{2+} during excitation of tonoplast-fee *Chara* cells. Protoplasma 117:62–67

Kikuyama M, Oda K, Shimmen T, Hayama T, Tazawa M (1984) Potassium and chloride effluxes during excitation of Characeae cells. Plant Cell Physiol 25:965–974

Kishimoto U (1968) Response of *Chara* internodes to mechanical stimulation. Ann Rep Biol Works, Fac Sci, Osaka Univ 16:61–66

Knight MR, Smith SM, Trewavas AJ (1992) Wind-induced plant motion immediately increases cytosolic calcium. Proc Natl Acad Sci USA 89:4967–4971

Kumon K, Tsurumi S (1984) Ion efflux from pulvinar cells during slow downward movement of the petiole of *Mimosa pudica* L. induced by photostimulation. J Plant Physiol 115:439–443
Malone M (1992) Kinetics of wound-induced hydraulic signals and variation potentials in wheat seedlings. Planta 187:505–510
Malone M, Stankovic B (1991) Surface potentials and hydraulic signals in wheat leaves following localized wounding by heat. Plant Cell Environ 14:431–436
Mertz SM Jr, Higinbotham N (1976) Transmembrane electropotential in barley roots as related to cell type, cell location, and cutting and aging effects. Plant Physiol 57:123–128
Meyer AJ, Weisenseel MH (1997) Wound-induced changes of membrane voltage, endogenous currents, and ion fluxes in primary roots of maize. Plant Physiol 114:989–998
Mimura T, Shimmen T (1994) Characterization of the Ca^{2+}-dependent Cl^- efflux in perfused *Chara* cells. Plant Cell Physiol 35:793–800
Oda K (1976) Simultaneous recording of potassium and chloride effluxes during an action potential in *Chara corallina*. Plant Cell Physiol 17:525–528
Qi Z, Kishigami A, Nakagawa Y, Iida H, Sokabe M (2004) A mechanosensitive anion channels in *Arabidopsis thaliana* mesophyll cells. Plant Cell Physiol 45:1704–1708
Rhodes JD, Thain JF, Wildon DC (1996) The pathway for systemic electrical signal conduction in the wounded tomato plant. Planta 200:50–57
Robin G (1979) *Mimosa pudica*: a model for the study of the excitability in plants. Biol Rev 54:135–153
Robin G (1985) Analysis of the variation potential induced by wounding in plants. Plant Cell Physiol 26:455–461
Robin G, Bonnemain J-L (1985) Propagation in *Vicia faba* stem of a potential variation induced by wounding. Plant Cell Physiol 26:1273–1283
Samejima M, Sibaoka T (1980) Changes in the extracellular ion concentration in the main pulvinus of *Mimosa pudica* during rapid movement and recovery. Plant Cell Physiol 21:467–479
Shiina T, Tazawa M (1987a) Ca^{2+}-activated Cl^- channel in plasmalemma of *Nitellopsis obtusa*. J Membr Biol 99:137–146
Shiina T, Tazawa M (1987b) Demonstration and characterization of Ca^{2+} channel in tonoplast-free cells of *Nitellopsis obtusa*. J Membrane Biol 96:263–276
Shiina T, Tazawa M (1988) Ca^{2+}-dependent Cl^- efflux in tonolast-free cells of *Nitellopsis obtusa*. J Membr Biol 106:135–139
Shimmen T (1988) Characean actin bundles as a tool for studying actomyosin-based motility. Bot Mag Tokyo 101:533–544
Shimmen T (1996) Studies on mechanoperception in characean cells: development of a monitoring apparatus. Plant Cell Physiol 37:591–597
Shimmen T (1997a) Studies on mechanoperception in characean cells: pharmacological analysis. Plant Cell Physiol 38:139–148
Shimmen T (1997b) Studies on mechanoperception in Characeae: effect of external Ca^{2+} and Cl^-. Plant Cell Physiol 38:691–697
Shimmen T (1997c) Studies on mechanoperception in Characeae: decrease in electrical membrane resistance in receptor potentials. Plant Cell Physiol 38:1298–1301
Shimmen T (2001) Electrical perception of "death message" in *Chara*: involvement of turgor pressure. Plant Cell Physiol 42:366–373
Shimmen T (2002) Electrical perception of "death message" in *Chara*: analysis of rapid component and ionic process. Plant Cell Physiol 43:1575–1584
Shimmen T (2003) Studies on mechanoperception in the Characeae: transduction of pressure signals into electrical signals. Plant Cell Physiol 44:1215–1224
Shimmen T (2005) Electrical perception of "death message" in *Chara*: analysis of K^+-sensitive depolarization. Plant Cell Physiol. 46: 1839–1847
Shimmen T (2006) Electrical perception of "death message" in *Chara*: characterization of K^+-induced depolarization. Plant Cell Physiol. 47:559–562
Shimmen T, MacRobbie EAC (1987) Characterization of two proton transport systems in the tonoplast of plasmalemma-permeabilized *Nitella* cells. Plant Cell Physiol 28:1023–1031

Shimmen T, Nishikawa S (1988) Studies on the tonoplast action potential of *Nitella flexlilis*. J Membr Biol 101:133–140
Shimmen T, Tazawa M (1977) Control of membrane potential and excitability of *Chara* cells with ATP and Mg^{2+}. J Membr Biol 37:167–192
Shimmen T, Tazawa M (1978) Intracellular chloride and potassium in relation to excitability of *Chara* membrane. J Membr Biol 55:223–232
Shimmen T, Tazawa M (1983) Control of cytoplasmic streaming by ATP, Mg^{2+} and cytochalasin B in permeabilized Characeae cell. Protoplasma 115:18–24
Shimmen T, Yokota E (2004) Cytoplasmic streaming in plants. Curr Opin Cell Biol 16:68–72
Shimmen T, Kikuyama M, Tazawa M (1976) Demonstration of two stable potential states of plasmalemma of *Chara* without tonoplast. J Membr Biol 30:249–270
Shimmen T, Mimura T, Kikuyama M, Tazawa M (1994) Characean cells as a tool for studying electrophysiological charcteristics of pant cells. Cell Struct Funct 19:263–278
Sibaoka T (1964) Response of plants to stimuli. In: Ashida J, Kawakita A, Yoshikawa H, Miyachi D, Okada I, Sakaguchi K, Tamiya H, Yamada T (eds) Seibutsu-no-hannousei (in Japanese). Kyoritsu-Shuppan, Tokyo, pp 133–166
Sibaoka T (1981) Ugoku-Shokubutsu (action plants) (in Japanese). Tokyo Daigaku-Shuppankai, Tokyo
Sibaoka T (1991) Rapid plant movement triggered by action potentials. Bot Mag Tokyo 104:73–95
Sibaoka T, Tabata T (1981) Electrotonic coupling between adjacent internodal cells of *Chara braunii*: transmission of action potentials beyond the node. Plant Cell Physiol 22:397–411
Stahlberg R, Cosgrove DJ (1994) Comparison of electric and growth responses to excision in cucumber and pea seedlings. I. Short-distance effects are a result of wounding. Plant Cell Environ 17:1143–1151
Stankovic B, Davies E (1996) Both action potential and variation potential induce proteinase inhibitor gene expression in tomato. FEBS Lett 390:275–279
Stankovic B, Zawadzki T, Davies E (1997) Characterization of the variation potential in sunflower. Plant Physiol 115:1083–1088
Staves MP, Wayne R (1993) The touch-induced action potential in *Chara*: inquiry into the ionic bases and the mechanoreceptor. Aust J Plant Physiol 20:471–488
Tazawa M, Kishimoto U, Kikuyama M (1974) Potassium, sodium and chloride in the protoplasm of Characeae. Plant Cell Physiol 15:103–110
Tazawa M, Kikuyama M, Shimmen T (1976) Electric characteristics and cytoplasmic streaming of Characeae cell lacking tonoplast. Cell Struct Funct 1:165–176
Tominaga Y, Shimmen T, Tazawa M (1983) Control of cytoplasmic streaming by extracllular Ca^{2+} in permeabilized *Nitella* cells. Protoplasma 116:75–77
Tominaga Y, Wayne R, Tung HYL, Tazawa M (1987) Phosphorylation-dephosphorylation is involved in Ca^{2+}-controlled cytoplasmic streaming in characean cells. Protoplasma 136:161–169
Wildon DC, Thain JF, Minchin PEH, Bugg IR, Reilly AJ, Skipper YD, Doherty HM, O'Donnell J, Bowles DJ (1992) Electrical signalling and systemic proteinase inhibitor induction in the wounded plant. Nature 360:62–65
Williamson RE, Ashley CC (1982) Free Ca^{2+} and cytoplasmic streaming in the alga *Chara*. Nature 296:647–651