United in Diversity: Mechanosensitive Ion Channels in Plants

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Abstract

Mechanosensitive (MS) ion channels are a common mechanism for perceiving and responding to mechanical force. This class of mechanoreceptors is capable of transducing membrane tension directly into ion flux. In plant systems, MS ion channels have been proposed to play a wide array of roles, from the perception of touch and gravity to the osmotic homeostasis of intracellular organelles. Three families of plant MS ion channels have been identified: the MscS-like (MSL), Mid1-complementing activity (MCA), and two-pore potassium (TPK) families. Channels from these families vary widely in structure and function, localize to multiple cellular compartments, and conduct chloride, calcium, and/or potassium ions. However, they are still likely to represent only a fraction of the MS ion channel diversity in plant systems.

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1. INTRODUCTION

How cells sense mechanical force is a long-standing question in biology. Mechanical signals such as touch, gravity, and osmotic pressure are critical to proper development, environmental stress response, and overall cellular health in a wide variety of prokaryotic and eukaryotic organisms and cell types. The perception of force can be mediated through the actions of integrins or focal adhesions, cytoskeletal reorganization, or nuclear deformation (reviewed in 32).

Alternatively, the application of intracellular or extracellular force can result in the deformation of cellular membranes, where the force is perceived by a specialized class of ion channels. Ion channels, membrane-spanning protein complexes that facilitate the flux of ions across the lipid bilayer, are responsible for a wide range of functions across all of life, including the production of action potentials in nerve cells (103), the maintenance of the ionic conditions required for metabolism in plants (134), and Ca^{2+} signaling in all cells (19). Interested readers are referred to two recent reviews on plant ion channel function (47, 122).

Ion channel: a gated macromolecular pore in a cell membrane that, once opened, permits ions to flow down their electrochemical gradient

MS: mechanosensitive

The flux of ions through a channel can be regulated by a variety of stimuli, including transmembrane (TM) voltage (9), ligand binding (59), light (23), and mechanical force (80). It is the latter stimulus that defines a diverse group of channels known as mechanosensitive (MS) ion channels,

also referred to as stretch-activated or force-gated channels. MS ion channels are present in all three domains of life (4, 60, 63), pointing to the fundamental requirement for mechanosensation in all cells.

Several recent reviews have described the wide variety of mechanical stimuli that land plants must sense and respond to during their life span (21, 87, 115). In addition, as many as 18 distinct MS ion channel activities (described in detail below) have been identified in plant membranes by patch-clamp electrophysiology, implying that mechanically gated ion channels play an important role in plant systems. In this review, we provide a general introduction to MS ion channel structure and function, outline the approaches used to study plant MS ion channels, and summarize current knowledge of the three families of plant MS ion channels, emphasizing their diverse structure, evolutionary history, and physiological roles.

Patch-clamp electrophysiology: a technique for measuring current across an isolated patch of membrane under conditions that maintain a particular transmembrane voltage

Protoplast: a bacterial, fungal, or plant cell from which the cell wall has been removed

Gating: a conformational change undergone by an ion channel in response to stimuli that creates an ion-permeable pore through the membrane

2. MECHANOSENSITIVE ION CHANNELS: TRANSDUCING FORCE INTO CURRENT

The electrical excitability of cells was first studied in the giant cells of Characean algae, prior to the adoption of the giant axons of squid as a model system in the 1930s (reviewed in 123). The advent of the patch-clamp technique, which permitted the study of individual channels in isolated cell membranes (see Section 4.2), made possible the first identification of MS ion channel activities in animal skeletal cells (40, 42). Shortly thereafter, MS ion channel activities were detected in tobacco (*Nicotiana tabacum*), broad bean (*Vicia faba*), and giant *Escherichia coli* protoplasts (33, 82, 107). The structures of two of the MS ion channels identified in those *E. coli* protoplasts have been solved at atomic resolution, providing the foundation for many elegant experimental and theoretical investigations into the molecular mechanism of MS ion channel activity (reviewed in 112).

An ion channel can be idealized as a two-state system, where it exists in either a closed (nonconducting) or open (conducting) state. The transition from a closed to an open state is referred to as gating. Once gated, an ion channel does not require additional energy to conduct a current; rather, ions move down their electrochemical gradient in either direction across the membrane through the channel pore (48).

For some classes of MS ion channels, increased membrane tension leads directly to gating. This behavior has been described by several biophysical models that address the energetic interactions at the membrane-protein interface (reviewed in 46). One proposed mechanism is the lipid-disordering model illustrated in **Figure 1*a***: An ion channel increases the free energy of the membrane in which it is embedded, as the lipids in that membrane must be disordered to conform to the shape imposed by the boundaries of the protein. With the addition of potential energy in the form of membrane tension, a conformational change in the channel that reduces the local deformation imposed on the membrane, while opening the channel pore, is favored (81, 116).

Another driving force for MS ion channel gating may be the thinning of the lipid bilayer under increased membrane tension (**Figure 1*b***). According to this model, membrane thinning results in a mismatch between the height of the channel's hydrophobic TM domain and the profile of the lipid bilayer, leading to the exposure of nonpolar side chains to the aqueous intra- or extracellular environment. A conformational change in the channel that maintains energetically favorable interactions between the TM domain and the lipid bilayer (such as rotating a TM helix within the plane of the membrane) is then coupled to the opening of the channel pore (79, 83). It is worth noting that the lipid-disordering and hydrophobic mismatch mechanisms are not mutually exclusive, and that there are likely many other mechanisms capable of driving intrinsic mechanosensitivity in ion channels (97, 99).

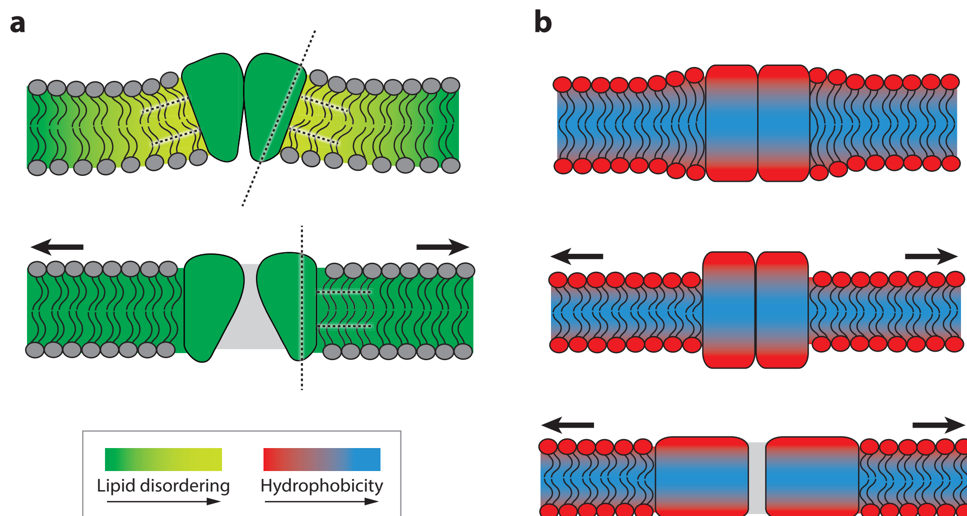


Figure 1

Models for mechanosensitive (MS) ion channel gating. (*a*) The lipid-disordering model. MS ion channels force the membrane to distort to establish favorable interactions with the channel (*top*). Lateral membrane tension (*arrows*) increases bilayer energy as the membrane structure is further altered. The open conformation of the channel is then favored because it reduces lipid disordering through a lower-energy interface with the membrane (*bottom*). The level of lipid disordering is indicated by yellow shading, and the conformational changes of the channel relative to the membrane are emphasized by dashed lines. (*b*) The hydrophobic mismatch model. Membrane bilayers create favorable interactions between the polar lipid heads and polar residues of an embedded protein (*top*). Lateral membrane tension (*arrows*) results in a thinner bilayer, disrupting some of these favorable interactions (*middle*). The open conformation of the channel, which has a shorter channel profile within the membrane, restores these interactions (*bottom*). The increasing hydrophobicity of regions is shown as a gradient from very hydrophilic (*red*) to very hydrophobic (*blue*).

In the two models shown in **Figure 1**, membrane tension is transmitted directly to the channel through the lipid bilayer. Alternatively, some MS ion channels—including those proposed to mediate hearing in the vertebrate inner-ear hair cells and gentle touch in *Caenorhabditis elegans*—are likely to be gated indirectly by tethering to other cellular components (29). Tension applied to a physical link between a channel and the extracellular matrix or the intracellular cytoskeletal system could directly stretch open the channel, reorient the channel in the lipid bilayer, or lead to lipid raft reorganization (2, 13, 46). A unifying theme in all of these models, however, is that the responsiveness of an MS ion channel to force depends on highly dynamic interactions with the lipid bilayer.

3. PHYSIOLOGICAL ROLES FOR MECHANOSENSITIVE ION CHANNELS

There are many ways in which an organism might employ a mechanosensor capable of transducing force into ion flux; many MS ion channels from animals have been studied in detail and shown to improve fitness during development or in a changeable environment. Studies of the physiological roles of MS ion channels in *C. elegans*, *Drosophila melanogaster*, and other metazoans inform our understanding of MS ion channels in plants, regardless of whether the channels are evolutionarily

PIEZO CHANNELS

Piezo channels, named for the Greek word for pressure, are believed to mediate the perception of mechanical stimuli in animal systems (reviewed in 95, 120, 129). mPiezo1 and mPiezo2 were identified in a tour de force RNA-silencing screen for the gene underlying mechanosensitivity in a mouse tissue culture cell line, and genes encoding Piezo homologs have been identified throughout the animal kingdom, in protists, in amoebae, and, surprisingly, in plants. Piezos are exceptionally large proteins, comprising 2,000–4,000 amino acids and 20–40 predicted transmembrane helices. Expressed in both sensory and nonsensory tissues, they are required for response to gentle touch and for vascular development in zebrafish and mice. They are further implicated in noxious touch response, cellular extrusion, and red blood cell volume regulation in flies, fish, mice, rats, and humans. Although heterologous expression of Piezo channels can confer mechanosensitivity on an insensitive cell, whether they require other cellular components or a specialized lipid environment for mechanosensitivity is not yet known. Mutations in human Piezo genes are associated with a number of diseases.

related. We thus briefly summarize what is known about MS ion channels in these species and then address their potential functions in plants.

3.1. MS Ion Channels in Animals

Several distinct families of MS ion channels are thought to underlie the senses of touch, pain, hearing, proprioception, and gravity sensation in animal systems. For example, response to light touch is mediated by the degenerin/epithelial sodium channel (Deg/ENaC) family in *C. elegans*. The transient receptor potential (TRP) family mediates nose touch and proprioception in *C. elegans* and hearing, nociception, and bristle touch in *D. melanogaster* (reviewed in 4). Two-pore potassium (TPK) MS ion channels are required for pressure-responsive vasodilation and also appear to regulate the pain threshold for cold and heat in mice (reviewed in 49). The recently identified Piezo family mediates diverse mechanosensory events in animals, from touch and pain in sensory neurons to intercellular communication and osmotic control (reviewed in 120; see also sidebar Piezo Channels).

Degenerin/epithelial sodium channel (DEG/ENaC): a family of cation-selective ion channels that mediate touch response in animals; they are especially well characterized in *C. elegans*

Transient receptor potential (TRP): a family of cation-selective candidate MS ion channels that potentially mediate multiple sensory pathways in animals

Two-pore potassium (TPK): a family of mechanically modulated, potassium-selective channels that localize to the plant vacuole and participate in ion homeostasis

3.2. MS Ion Channels in Plants

Plants sense and respond to many of the same mechanical stimuli as animals, including touch, gravity, and osmotic stress (16, 87, 115). They also respond to unique signals associated with developmental processes, such as lateral root emergence, pollen tube growth, cell wall damage, and plant-pathogen interactions (3, 54, 74). In many of these cases, applying a mechanical stimulus leads to a rapid burst of ion flux, and it has long been speculated that this correlation may be attributed to the action of MS ion channels in the stimulated cells, in part because of the speed of the response (reviewed in 34, 53, 87).

The flux of Ca^{2+} in particular has been implicated in various mechanosensory pathways. For example, gravity stimulation (introduced by rotating a root or shoot 90°) is associated with membrane depolarization, the rapid influx of Ca^{2+} ions, and subsequent alkalinization of the cells in the root cap (reviewed in 115). Ca^{2+} influx is also associated with touch stimulus (reviewed in 34), osmotic stress (109), and bending (86), consistent with the action of a mechanically gated calcium channel in these processes. After influx, Ca^{2+} could serve as a second messenger in a large number

of downstream events—some of which, such as the activation of calmodulin and calmodulin-like proteins, are also implicated in mechanotransduction (16).

Trinitrophenol (TNP): a negatively charged amphipath that inserts into the outer bilayer of a membrane and induces curvature; also called picric acid

4. APPROACHES USED TO STUDY MECHANOSENSITIVE ION CHANNELS IN PLANTS

4.1. Pharmacological Inhibition or Activation

Evidence that MS ion channels are an integral part of a particular mechanosensory process can be obtained through pharmacological treatments with known inhibitors or activators of MS ion channels. The Ca^{2+} influx associated with mechanical stimulation can be inhibited by lanthanides (26, 85, 90), ruthenium red (69), or cytoskeletal inhibitors (25). However, these treatments are often nonspecific. For example, the commonly used lanthanide Gd^{3+} blocks a wide variety of channels, not solely Ca^{2+}-selective or MS ion channels (71). Furthermore, Gd^{3+} can indirectly inhibit the action of nonselective MS ion channels by reducing overall membrane fluidity (31, 76). On the other hand, the chemical trinitrophenol (TNP), which increases curvature and tension when applied to membranes (81), behaves as an MS ion channel activator and can induce Ca^{2+} flux or lower the threshold for mechanical stimulation (37, 90, 104). Although sensitivity to one of these pharmacological agents can provide evidence that MS ion channels are involved in a particular response, confirmation will likely require knowing the molecular identity of the channels involved and characterizing their channel properties through the methods described below.

4.2. Electrophysiology

The gold standard technique for the analysis of MS ion channels is patch-clamp electrophysiology. Patch clamping involves producing a high-resistance seal between the glass of a micropipette tip and a small patch of membrane containing the channels of interest (106). The pipette can remain attached to the cell with an intact patch (cell-attached), remain attached to the cell with a ruptured patch (whole-cell), or be completely removed from the cell along with the patch (excised). In all of these configurations, membrane tension is increased by introducing positive or negative pressure through the patch pipette, and the resulting current across the membrane is recorded over time. This technique has been used to identify and characterize plant MS ion channels in their native membranes or heterologously expressed in *Xenopus* oocytes, as described in more detail below. Although patch clamping allows the identification of individual MS ion channels—and, in the excised patch configuration, control over the ionic conditions on both sides of the membrane—a drawback especially relevant to plant systems is that it requires isolating cells from the tissue and removing the cell wall.

4.3. Genetics

Molecular genetic approaches in *Arabidopsis thaliana* and other model plant systems have added another dimension to the study of MS ion channels in recent years. Although a classical genetic screen has not yet successfully been used to identify an MS ion channel, reverse genetics motivated by either phylogenetics or a functional assay has identified several candidates (44, 75, 90). Once a candidate gene is identified, the protein can be heterologously expressed and tested for MS ion channel activity in cell survival or electrophysiological assays. Genetic ablation or overexpression of candidate MS ion channel genes in planta can be powerful tools for characterizing channels in their native systems.

ESCHERICHIA COLI MSCS

The mechanosensitive channel of small conductance (MscS) of *E. coli* was among the first mechanosensitive channels to be identified and is now one of the best understood in any system (reviewed in 11, 46, 63). MscS is a weakly anion-preferring channel with a conductance of ~1 nanosiemens, and contributes to cellular survival of hypoosmotic shocks ranging from 500 to 1,000 milliosmoles. MscS activity can be reconstituted with only recombinant protein and lipids, indicating that it is gated directly through membrane tension. The C-terminal domain also undergoes a structural rearrangement upon gating and has been proposed to help regulate the composition of osmolytes available to pass through the channel pore. Five crystal structures of prokaryotic MscS homologs in conducting and nonconducting conformations, molecular dynamic simulations, and a slew of structure-function studies support several models for the MscS gating mechanism. Taken together, these studies form a solid foundation for future investigations into the structure, biophysical mechanism, and physiological function of MscS homologs in plants.

5. CRITERIA FOR ASSIGNMENT AS A MECHANOSENSITIVE ION CHANNEL

Establishing that a particular gene encodes the primary force transducer in an MS response, as opposed to an accessory or downstream component of this response, is a challenging endeavor. The following criteria have previously been established: (*a*) The observed MS response displays the proper expression and localization, (*b*) the channel is required for the response but not for the normal development of the cell or tissue in which the response occurs (unless the MS response being measured is the development of the tissue itself), (*c*) there is evidence of MS gating in isolation or in heterologous systems, and (*d*) structural alterations of the protein produce changes in the MS response and channel behavior (4, 18, 89). Meeting all four criteria to definitively categorize MS ion channels can be difficult, especially if MS ion channels function as heteromultimers or with other cellular structures.

Although none of the MS ion channel candidates so far identified in plants fulfill all four of these criteria, we refer to them here as MS ion channels in consideration of the strong evidence that does exist in favor of this interpretation in each case. **Table 1** summarizes relevant information about the three families of plant MS ion channels that have so far been identified: the MscS-like (MSL), Mid1-complementing activity (MCA), and TPK channels. As summarized below, channels from these three families vary widely in terms of structure and function, localize to multiple cellular compartments, and conduct diverse subsets of ions.

MscS-like (MSL): a family of MS ion channels that protect cells or organelles from osmotic stress in bacteria, archaea, fungi, and plants; they may have additional physiological functions

Mid1-complementing activity (MCA): a family of plasma membrane–localized MS calcium channels that mediate osmotic stress response and calcium homeostasis in plants

Conductance: a measurement of the ease with which current flows through an ion channel at a given voltage, measured in siemens (S)

Hypoosmotic shock: a rapid decrease in the osmolarity of the media for a membrane-bound cell or organelle, resulting in water influx into the cell

6. MSCS-LIKE CHANNELS

The first family of putative plant MS ion channels was identified based on the channels' similarity to the *E. coli* mechanosensitive channel of small conductance (MscS), a well-established model system for the study of MS ion channels (11, 84; see also sidebar *Escherichia coli* MscS). MscS serves as an emergency release valve under conditions of hypoosmotic shock in *E. coli* (11, 70). Genes predicted to encode homologs of MscS are found throughout bacterial and archaeal genomes; in some protist genomes, including pathogenic protozoa; and in all plant genomes so far examined (6, 43, 61, 62, 78, 101, 102, 127). MscS homologs have not yet been identified in animal genomes.

Figure 2*a* presents the predicted evolutionary relationship among representative members of the MscS superfamily, using the ~100-amino-acid domain conserved among the members of the MscS superfamily (100). This sequence maps to the pore-lining helix and the upper part of the

Table 1 Plant mechanosensitive (MS) ion channels

Protein	Gene	Organism	Mutant/silenced phenotype	Overexpression phenotype	Subcellular localization	Expression-associated MS ion channel characteristics	Activity in heterologous systems	Key reference(s)
MscS-like (MSL)								
MSL1	At4g00290	*Arabidopsis thaliana*			Mitochondria[a]		Survival of hypoosmotic shock in *E. coli*	43
MSL2	At5g10490	*Arabidopsis thaliana*	*msl2* null mutants show defective leaf shape; *msl2 msl3* double mutants have enlarged chloroplasts and enlarged, round nongreen plastids; *msl2 msl3* double mutants have chloroplasts that exhibit multiple division rings.		Plastid envelope, poles			44, 55, 118, 126
MSL3	At1g58200	*Arabidopsis thaliana*	*msl2 msl3* double mutants have enlarged chloroplasts and enlarged, round nongreen plastids; *msl2 msl3* double mutants have chloroplasts that exhibit multiple division rings.		Plastid envelope, poles		Survival of hypoosmotic shock in *E. coli*	44, 118, 126
MSL4	At1g53470	*Arabidopsis thaliana*	*msl4 msl5 msl6 msl9 msl10* quintuple mutants lack MS ion channel activity in root protoplasts.		Plasma membrane[a]			45
MSL5	At3g14810	*Arabidopsis thaliana*	*msl4 msl5 msl6 msl9 msl10* quintuple mutants lack MS ion channel activity in root protoplasts.		Plasma membrane[a]			45

MSL6	At1g78610	*Arabidopsis thaliana*	*msl4 msl5 msl6 msl9 msl10* quintuple mutants lack MS ion channel activity in root protoplasts.		Plasma membrane[a]			45
MSL9	At5g19520	*Arabidopsis thaliana*	*msl9* mutants lack an ~45-pS MS ion channel activity in root protoplasts; *msl9 msl10* double mutants lack an ~50-pS MS ion channel activity in root protoplasts.		Plasma membrane, ER			45
MSL10	At5g12080	*Arabidopsis thaliana*	*msl10* mutants lack an ~140-pS MS ion channel activity in root protoplasts; *msl9 msl10* double mutants lack an ~50-pS MS ion channel activity in root protoplasts.	Cell death, increased H_2O_2 accumulation, induction of *SAG12*, *OSM34*, *DOX1*, *PERX34*, and *KTI1*	Plasma membrane, ER	~100-pS conductance in *Xenopus* oocytes, with a moderate preference for anions		45, 77, 117
Mid1-complementing activity (MCA)								
MCA1	At4g35920	*Arabidopsis thaliana*	The roots of *mca1* mutants are less efficient at penetrating hard agar and do not induce lignin deposition or alter carbohydrate gene expression patterns in response to isoxaben; *mca1 mca2* double mutants are hypersensitive to $MgCl_2$ and show developmental delays.	Increased Ca^{2+} uptake in seedling roots, increased Ca^{2+} influx (measured as aequorin signal) in response to hypoosmotic shock and TNP treatment, high *TCH3* expression level	Plasma membrane	~15- or ~35-pS-conductance mechanically gated channel in *Xenopus* oocytes	Survival and Ca^{2+} uptake in response to challenge with mating pheromone in *S. cerevisiae*; stretch-activated Ca^{2+} influx in CHO cells	24, 36, 90, 128, 130

(*Continued*)

Table 1 (*Continued*)

Protein	Gene	Organism	Mutant/silenced phenotype	Overexpression phenotype	Subcellular localization	Expression-associated MS ion channel characteristics	Activity in heterologous systems	Key reference(s)
MCA2	At2g17780	*Arabidopsis thaliana*	*mca2* mutants show a reduction in Ca^{2+} uptake; *mca1 mca2* double mutants are hypersensitive to $MgCl_2$ and show developmental delay.		Plasma membrane		Survival and Ca^{2+} uptake in response to challenge with mating pheromone in *S. cerevisiae*	130
NtMCA1	AB622811	*Nicotiana tabacum*		Increased Ca^{2+} uptake in cultured tobacco cells, increased *NtERF4* expression	Plasma membrane, punctate signal		Survival and Ca^{2+} uptake in response to challenge with mating pheromone, Ca^{2+} uptake in response to hypoosmotic shock in *S. cerevisiae*	66
NtMCA2	AB622812	*Nicotiana tabacum*		Increased Ca^{2+} uptake in cultured tobacco cells, increased *NtERF4* expression	Plasma membrane, punctate signal		Survival and Ca^{2+} uptake in response to challenge with mating pheromone, Ca^{2+} uptake in response to hypoosmotic shock in *S. cerevisiae*	66

OsMCA1	Os03g0157300	*Oryza sativa*	*OsMCA1*-silenced lines exhibit slower growth as well as reduced aequorin luminescence in response to hypoosmotic shock or to the membrane-distorting agent TNP.	Increased Ca^{2+} uptake in cultured rice cells	Plasma membrane			65
Two-pore potassium (TPK)								
TPK1	At5g55630	*Arabidopsis thaliana*	*tpk1* mutants lack an instantaneous tonoplast K^+ current in all shoot cell types and show modest sensitivity to high and low K^+ in the media, slow guard cell–closing kinetics in response to ABA, and slow germination, especially in the presence of ABA.	Resistance to high and low K^+ in the media, fast guard cell–closing kinetics in response to ABA, fast germination	Vacuolar membrane	Instantaneous K^+-selective channel activity that increases with membrane tension	Complements a K^+-uptake-deficient *E. coli* mutant	39, 52, 75
OsTPK1a	Os03g541002	*Oryza sativa*			Vacuolar membrane	Instantaneous K^+-selective channel activity that increases with membrane tension		51, 75
HvTPK1	EU926490	*Hordeum vulgare*			Vacuolar membrane	Instantaneous K^+-selective channel activity that increases with membrane tension		12, 75

Blank cells indicate that no data are available. Abbreviations: ABA, abscisic acid; CHO, Chinese hamster ovary; *E. coli*, *Escherichia coli*; ER, endoplasmic reticulum; pS, picosiemens; *S. cerevisiae*, *Saccharomyces cerevisiae*; TNP, trinitrophenol.

[a]Predicted.

a

Plants, group I
V. vinifera 00026926001
A. thaliana MSL1
P. patens 1s314_12
C. papaya 11.45
Os02g45690 O. sativa
2G125494 Z. mays
5g19160 B. distachyon

MSC1 C. reinhardtii
MSC2 C. reinhardtii
MSC3 C. reinhardtii

Bacteria
YbdG E. coli
Ss-bCNGa Synechocystis sp. PCC6803
MscS H. pylori
MscS E. coli
MscS T. tengcongensis
MscCG C. glutamicum

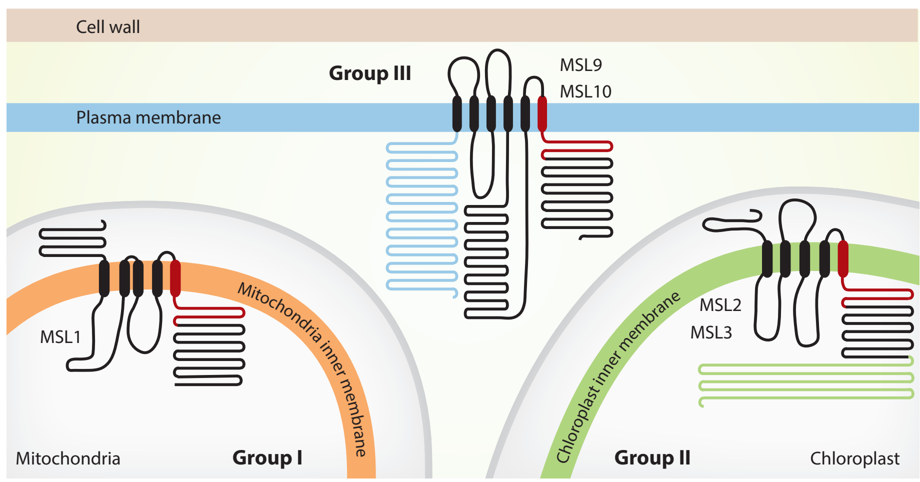
Plants, group II
C. papaya 55.26
A. thaliana MSL2
P. patens 1s79_156
A. thaliana MSL3
P. trichocarpa 002G105900
V. vinifera 00015105001
B. distachyon 1g15920.1
O. sativa Os04g48940
Z. mays 2G028914

Protozoa and fungi
P. falciparum AAN35680
T. gondii TGME49_034410
D. discoideum DDB_G0277253
S. pombe MSY1
S. pombe MSY2

Plants, group III
MSL8 A. thaliana
20.126 C. papaya
004G178900 P. trichocarpa
Os02g44770 O. sativa
3g51250 B. distachyon
1s2_432 P. patens
1g46387.1 B. distachyon
Os06g10410 O. sativa
2G005013 Z. mays
V. vinifera 00002410001
A. thaliana MSL10
A. thaliana MSL9
C. papaya 126.38

4.0 amino acid substitutions per site

b



cytoplasmic vestibule of MscS (7). A number of highly conserved motifs within this domain are important for function in bacterial and plant channels (6, 22, 55). Land plant MscS homologs fall into three phylogenetic groups (I–III), which also correspond to three different subcellular localizations (see below).

Plastid: a plant-specific endosymbiotic organelle in which photosynthesis, biosynthesis, and/or storage of cell metabolites take place

6.1. A Diverse Family of MS Ion Channels

Outside of the conserved MscS domain, MscS family members are highly divergent in their topology and domain structure. Among others, domains associated with cyclic nucleotide, Ca^{2+}, or K^{+} binding are appended to the basic MscS topology (78, 117, 127). In addition, plant MSL proteins localize to multiple cellular compartments, providing further evidence that they serve a diverse set of functions within the cell (**Figure 2*b***).

Group I and group II MSL proteins are predicted (and in some cases have been shown) to localize to mitochondria and plastids, respectively (43, 44). Both groups are predicted to contain five TM helices, the last helix corresponding to the pore-lining domain of MscS, and a C terminus that is located in the stroma or matrix. Group III MSL proteins are predicted or have been shown to localize to the plasma membrane (43, 45) and to contain six TM helices, again with the most C-terminal TM segment corresponding to the pore-lining domain of MscS. They also have a large cytoplasmic N terminus, a cytoplasmic loop of variable length between TM regions 4 and 5, and a cytoplasmic C terminus.

The *A. thaliana* genome encodes ten *MSL* genes (**Table 1**), and they show a range of expression patterns, including root- and flower-specific expression (43). At the protein level, most *A. thaliana* MSLs can be grouped into pairs based on sequence homology. MSL2 and MSL3 are 50% identical at the amino acid level, MSL4 and MSL5 are 68% identical, and MSL7 and MSL8 are 71% identical and located in tandem on the chromosome (43). The existence of highly similar pairs of MSLs in the *A. thaliana* genome may indicate functional redundancy (as with MSL2 and MSL3; see below) but may also have permitted the evolution of unique characteristics (as with MSL9 and MSL10; see below).

Figure 2

Phylogenetic relationships, subcellular localizations, and topologies of MscS-like (MSL) channels. (*a*) The inferred phylogeny of 43 members of the MscS superfamily, presented as an unrooted radial tree. Sequences were identified by Phytozome BLAST analysis (**http://www.phytozome.net**) or inclusion in previous analyses (15, 43, 67, 93, 94, 101, 118, 133). The MSL region of each protein was identified by InterProScan (57) and aligned using ClustalW (114) with a gap-opening penalty of 3.0 and a gap-extension penalty of 1.8. The evolutionary history was inferred using the neighbor-joining (105) method with a JTT distance matrix (56) using MEGA6 software (113). The reliability of the tree was determined via bootstrapping ($n = 1{,}000$ replicates) (35), and branches with bootstrap values of less than 50% were collapsed. The phylogenetic origin or cluster is indicated in the colored areas. The sequences used in this analysis and their UniProt accession numbers, Arabidopsis Information Resource (TAIR) accession numbers, or Phytozome accession numbers are as follows: *Escherichia coli* MscS (P0C0S1), YbdG (P0AAT4); *Synechocystis* sp. PCC6803 bCNGa (M1ME31); *Helicobacter pylori* MscS (E1Q2W1); *Corynebacterium glutamicum* MscCG (P42531); *Thermoanaerobacter tengcongensis* MscS (Q8R6L9); *Toxoplasma gondii* (B6KM08); *Plasmodium falciparum* (Q8IIS3); *Dictyostelium discoideum* (Q54ZV3); *Schizosaccharomyces pombe* MSY1 (O74839), MSY2 (O14050); *Chlamydomonas reinhardtii* MSC1 (A3KE12), MSC2 (A8HM43), MSC3 (A8HM47); *Arabidopsis thaliana* MSL1 (At4g00290), MSL2 (At5g10490), MSL3 (At1g58200), MSL8 (At2g17010), MSL9 (At5g19520), MSL10 (At5g12080); *Populus trichocarpa* (Pt002G105900, Pt004G178900); *Zea mays* (GRMZM2G125494, GRMZM2G028914, GRMZM2G005013); *Oryza sativa* (Os02g45690, Os04g48940, Os06g10410, Os02g44770); *Brachypodium distachyon* (Bradi1g15920, Bradi5g19160, Bradi3g51250); *Vitis vinifera* (Vv00015105001, Vv00026926001, Vv00002410001); *Physcomitrella patens* (Pp1s79_156, Pp1s314_12, Pp1s2_4320); *Carica papaya* (supercontig_55.26, supercontig _22.80, supercontig _126.38, supercontig_20.126). (*b*) Predicted topology and subcellular localization of representative MSLs from *A. thaliana*. Topologies were drawn according to predictions on Aramemnon (**http://aramemnon.botanik.uni-koeln.de**). The regions of highest homology to *E. coli* MscS found in groups I, II, and III are shown in red; the group II–specific C-terminal extension is shown in green; and the group III–specific N-terminal region is shown in blue.

6.2. Evidence That MSLs Are MS Ion Channels

Chloroplast: a plastid specialized for photosynthesis

Filamentous temperature-sensitive Z (FtsZ): a GTPase that forms filaments required for fission in bacteria and plastids

It is currently accepted that MS ion channel activity has been retained among most members of the MscS superfamily (for one exception, however, see 15). All six MscS family members in *E. coli* are capable of producing tension-gated activities in giant spheroplasts (28, 70, 72, 108), as is MSC1 from *Chlamydomonas reinhardtii* (92) and MSY1 from fission yeast (*Schizosaccharomyces pombe*) (93).

Several lines of evidence support the hypothesis that MSLs form functional MS ion channels in *A. thaliana*. Plastid-localized MSL3 was able to partially rescue the susceptibility to hypoosmotic shock of an *E. coli* strain missing three major MS ion channels (44). More direct evidence was obtained for the endoplasmic reticulum– and plasma membrane–localized channels MSL9 and MSL10, which are genetically required for the primary MS ion channel activity detected by whole-cell electrophysiology in root protoplasts (45). A characterization of the conductance of MS ion channel activities present in root protoplasts from single *msl9*, single *msl10*, or double *msl9 msl10* mutants suggests that MSL9 and MSL10 can form a heteromeric channel with a conductance of ∼50 picosiemens (pS), whereas MSL9 and MSL10 homomeric channels have conductances of ∼45 pS and ∼140 pS, respectively (45, 98). The ability to form homo- and heteromeric channels with distinct properties, in combination with overlapping tissue-specific expression patterns for multiple *MSL* genes, could produce a range of MS responses across different tissues in plants.

In agreement with the in planta electrophysiology described above, it was recently shown that MSL10 is associated with an ∼100-pS MS ion channel activity when expressed heterologously in *Xenopus* oocytes (77). MSL10 channel activity in oocytes has a slight (sixfold) preference for anions and closes at lower tensions than it opens. MSL10 meets three of the four criteria for a bona fide MS ion channel: (*a*) *MSL10* is expressed in root cells, where (*b*) it is required for the wild-type MS ion currents but not for the normal development of the tissue, and (*c*) expression of *MSL10* in *Xenopus* oocytes confirms that it can form a functional MS ion channel in a heterologous system. However, it has not yet been determined that structural changes (such as point mutations in the putative pore-lining domain) alter its mechanosensitivity.

6.3. MSLs Serve as Osmotic Conduits in the Plastid Envelope

Of all MscS homologs in plants, we know the most about those that localize to the plastid envelope. Originally, it was surprising to find homologs of a protein known to protect a bacterial cell from environmental osmotic shock targeted to intracellular organelles. However, all evidence now suggests that group II MSLs serve a role related to (yet distinct from) the emergency release valve function. Consistent with bioinformatic predictions, *A. thaliana* MSL2 and MSL3 localize to the plastid envelope, likely to the inner membrane, and are observed in foci at the plastid poles (44, 126). Immunofluorescence of algal MSC1 similarly revealed a complex localization pattern of punctate spots within both the chloroplast and the cytoplasm (92).

Plants harboring lesions in *MSL2* and *MSL3* show numerous whole-plant and subcellular defects, but the most striking phenotype is the presence of greatly enlarged and spherical nongreen plastids in the epidermis and root (small, ovoid plastids are seen in wild-type epidermal cells) (44). These defects in plastid size and shape can be rescued by increasing the osmolarity of the cytoplasm relative to the plastid through a variety of genetic and environmental manipulations (118), strongly suggesting that nongreen plastids experience hypoosmotic stress under normal conditions within the cytoplasm and that MSL2 and MSL3 function redundantly to relieve this stress.

In photosynthetic tissues, *msl2 msl3* double mutants have fewer and larger chloroplasts than the wild type, possibly as a result of the multiple filamentous temperature-sensitive Z (FtsZ) rings observed in the chloroplasts of this mutant (126). As MSL2– and MSL3–green fluorescent protein (GFP) fusion proteins colocalize with the plastid division protein AtMinE (44), it is possible that

MSL2 and MSL3 interact with the plastid division machinery to influence division site selection; it is equally plausible that the defect in plastid division in *msl2 msl3* mutants derives from altered stromal ion homeostasis or a mechanical inability to constrict the FtsZ ring (125). A function for MS ion channels in division is evolutionarily conserved, as *E. coli* mutants lacking several key MS ion channels also exhibit defects in division site selection when exposed to the division inhibitor cephalexin (126). Chloroplast-localized MSC1 of *Chlamydomonas* is required for chloroplast integrity; whether the mechanism behind this defect is the same as in *msl2 msl3* mutants is not clear (92).

Single *msl2* and double *msl2 msl3* mutants also exhibit several whole-plant phenotypes, including dwarfing, rumpled leaf surfaces, thicker leaf lamina, and variegation (44, 55, 126). Although the source of these phenotypes remains under investigation, at least some of them are likely to be developmental responses to plastid osmotic stress. Plastid osmotic stress in these mutants leads to the activation of dehydration stress responses such as the accumulation of proline and the production of ABA, even in the absence of any extracellular osmotic stress (124). MSL2 and MSL3 appear to function partially redundantly to relieve plastid osmotic stress. Although a null *msl2* allele produces developmental defects even in the wild-type *MSL3* background (55), all mutant phenotypes are exacerbated in the *msl2 msl3* double mutant (44, 118, 124, 126). There is currently no null *msl3* allele, and exactly how the functions of MSL2 and MSL3 overlap and diverge remains to be established.

6.4. MSLs at the Plant Plasma Membrane

In contrast to the group II MSLs, establishing a role for group III MSLs in plants has been a challenge. None of the obvious assays (touch, gravity, osmotic shock, etc.) produce phenotypes distinguishable from the wild type, even in an *msl4 msl5 msl6 msl9 msl10* quintuple null mutant (45, 115). This may be surprising, given that MscS homologs MSY1 and MSY2, which localize to the endoplasmic reticulum of *S. pombe*, play an essential role in protecting yeast cells from hypoosmotic shock (93). This could be due to redundant mechanosensory pathways, or because this class of MSLs is required for plants to survive stressful conditions not easily replicated in the laboratory. Consistent with the latter interpretation, recent evidence points to a role for MSL10 in one or more stress-induced cell death signaling pathways. Both transient and stable MSL10 overexpression lead to dwarfing, H_2O_2-associated cell death, and the induction of cell death–associated gene expression (117).

6.5. Beyond the Paradigm of Emergency Release Valves

Although MscS functions as an emergency release valve in *E. coli*, it has become clear that it and other members of the MscS superfamily serve multiple complex roles in both prokaryotes and eukaryotes (reviewed in 10, 22, 78, 127). Based on the diverse localization, topology, and domain structure observed within the MscS superfamily, we have previously suggested that (*a*) some MSL channels may respond to osmotic stress other than that provided by the extracellular environment, (*b*) some may be regulated by mechanisms other than membrane tension, and (*c*) some might even have functions that are completely separable from their role in mediating ion flux (46).

Experimental support for these three ideas in *A. thaliana* MSLs has accumulated over the past decade. For example, (*a*) the osmotic swelling of *msl2 msl3* mutant plastids illustrates that the environment of the cytoplasm can be as osmotically stressful to organelles as the extracellular environment is to a bacterial cell, and that MSL channels can serve to protect organellar membranes from this stress during normal growth and development (44, 118). Furthermore, (*b*) there is evidence that group I, II, and III MSLs may be regulated by phosphorylation in addition to membrane tension. Multiple proteomic studies have identified phosphopeptides that map to MSL1, MSL3, MSL4,

MSL5, MSL6, MSL9, and MSL10 [summarized in the Arabidopsis Protein Phosphorylation Site Database (PhosPhAt) at **http://phosphat.uni-hohenheim.de**]. At least some of these modifications are likely to be functionally relevant, as the cell death signaling function of MSL10 can be controlled by mutating the phosphorylated residues in its soluble N-terminal domain (117), and MSL9 is a direct target of the drought-associated kinase SnRK2.6 (121). Finally, (*c*) at least one MSL does indeed have a function that is separable from ion flux, as the cell death signaling function of MSL10 requires only its soluble N-terminal domain, which is unique to MSL10 and its orthologs in other plant species and does not form a channel on its own (117). We anticipate that future studies in *A. thaliana* and other model systems will uncover a multiplicity of physiological functions and regulatory mechanisms for MSL channels.

Chinese hamster ovary (CHO) cells: a commonly used cell line for protein expression and tissue culture work

7. MID1-COMPLEMENTING ACTIVITY CHANNELS

Although MSLs are essential to organelle osmoregulation and likely play complex roles at the plasma membrane and endoplasmic reticulum, they are essentially nonselective ion channels. Thus, their discovery and characterization still left open the identity of the elusive calcium channels thought to be associated with mechanical signaling, as reviewed above. Soon, however, candidates for such channels were provided by the discovery of MCAs, a novel land plant–specific family of membrane-associated proteins. Only one or two family members are present in each plant genome, and homologs have not been found in algae or animals (66). Sequence conservation is not restricted to a single domain but rather is distributed along the length of the protein.

7.1. MCA Protein Function Is Tightly Correlated with Ca^{2+} Influx

The founding member of the MCA family, *A. thaliana* MCA1, was identified in a functional screen for cDNAs capable of rescuing the *mid1* mutant strain of *Saccharomyces cerevisiae* (90). Mid1 is a stretch-activated MS ion channel required for Ca^{2+} influx and cell survival after exposure to mating pheromone (58). *A. thaliana* MCA2 and *N. tabacum* MCA1 and MCA2 were identified based on homology to *A. thaliana* MCA1 and are also capable of promoting the survival of *mid1* yeast in the mating factor assay (66, 130).

MCA proteins from *A. thaliana*, *N. tabacum*, and rice appear to serve similar roles in all three organisms; for an overview of these genes and their characteristics, see **Table 1**. MCA-mediated activity responds to stimuli associated with increased membrane tension but also appears to contribute to Ca^{2+} homeostasis in the absence of stress (65, 66, 90, 130). Taken together, the current data support a model wherein MCA proteins either are themselves MS calcium channels or are closely associated with the activity of an MS calcium channel.

Most MCA-GFP fusion proteins localize to the plasma membrane of plant cells, often in a punctate pattern (65, 90, 91, 130). When expressed in yeast, MCA1 fractionates with plasma membrane proteins and behaves like an intrinsic membrane protein in solubility tests (90, 91). The overexpression of MCA proteins is associated with increased influx of Ca^{2+} into plant roots, plant tissue culture cells, Chinese hamster ovary (CHO) cells, or yeast cells, either in the absence of stimulus or transiently in response to hypoosmotic shock, cell stretching, or treatment with the membrane-distorting lipid TNP (65, 66, 90, 130). Additionally, increased expression of the touch-inducible genes *TCH3* (in *A. thaliana*) and *ERF3* (in *N. tabacum*) is correlated with the overexpression of MCA proteins (14, 66, 90, 96).

MCA genes are expressed broadly in a variety of tissues (65, 90, 130), and *MCA* T-DNA insertion mutants in *A. thaliana* and *OsMCA1*-silenced lines in rice show growth defects and late flowering (65, 130). *AtMCA1* and *AtMCA2* have partially divergent functions; *mca1* but not *mca2*

mutants show defects in root entry into hard agar (90, 130), whereas *mca2* but not *mca1* mutants are defective in Ca^{2+} uptake in *A. thaliana* roots (130). The *mca1 mca2* double mutant exhibits both of these phenotypes as well as growth that is hypersensitive to Mg^{2+} (probably owing to competition with Ca^{2+} for uptake) (130). Further evidence that MCAs are involved in signaling in response to membrane tension comes from studies of the cellular response to treatment with the cell wall biosynthesis inhibitor isoxaben, which leads to cellular swelling (68). MCA1 is required for the accumulation of lignin and altered expression pattern of carbohydrate metabolism genes that are observed in wild-type cells treated with isoxaben (24, 41, 128).

Isoxaben: an herbicide that prevents the incorporation of glucose into cell walls by inhibiting cellulose synthase subunits

Open probability: the ratio of channels that are open to those that are closed in a particular population

7.2. Structural Features of MCAs

Surprisingly, MCAs resemble neither Mid1 nor any known ion channels or membrane-bound transporters. They do encode three recognizable motifs, including an EF-hand-like motif at the N terminus, a coiled-coil motif, and a Plac8 motif at the C terminus (64). The membrane-spanning domains and topology of MCAs are still under investigation. Unpublished data indicate that MCAs harbor a single TM helix at the extreme N terminus (H. Iida, personal communication). Deleting this TM helix disrupts MCA1 and MCA2 function in yeast cells, as does changing a single conserved aspartic acid within it to asparagine (D21N) (91). Gel migration, gel filtration, and cross-linking studies have indicated that MCA1 and MCA2 form homotetramers (91, 110). Cryo-electron microscopy followed by single-particle reconstruction of purified MCA2 complexes revealed a tear-shaped structure, consistent with the complex forming a single narrow TM spanning region and a larger cytoplasmic domain (110).

Like MSL10, MCA-associated channel activity has been characterized in *Xenopus* oocytes. Through the use of the cell-attached patch-clamp method, a statistically significant increase in current in response to negative pressure was observed in oocytes expressing MCA1 or MCA2 compared with those expressing the plant potassium channel KAT1 or those injected with water (36). In addition, single-channel activities of ∼15 pS and ∼35 pS were occasionally detected in MCA1-expressing (but not in water-injected) oocytes in response to negative pressure. These data support the model derived from other studies that MCAs form mechanically gated Ca^{2+}-permeable ion channels, but still fall short of establishing this unequivocally by introducing a mutation that alters channel behavior. As the two single-channel activities attributed to MCA1 appear to be rare [detected in 16 and 5 patches out of 71, respectively (36)], it is possible that association with a plant-specific component is required for full activity.

8. TWO-PORE POTASSIUM CHANNELS

A third group of plant MS ion channels includes channels related to those in the mammalian TPK family (also designated K_{2P}; see **Table 1** for a summary of their relevant properties). As is evident from their name, TPKs possess two pore domains and are K^+ selective. TPK activity is pH sensitive and voltage independent and can often be activated by increased $[Ca^{2+}]_{cyt}$ (30). Membrane tension modulates the open probability of several mammalian TPKs (8, 17), and they have been proposed to play a variety of mechanosensory roles in cardiomyocytes, the smooth muscle of the stomach and intestines, and pain perception (49).

In plants, a subset of TPK proteins are localized to the vacuolar membrane, where they most likely encode the VK (vacuolar K^+ selective) channel activities previously characterized in guard cells (summarized in 47, 119). AtTPK1, a vacuolar membrane–localized TPK from *A. thaliana*, is required for normal stomatal closure kinetics, K^+ homeostasis in multiple tissue types, and efficient seed germination (39). The cytoplasmic domains of many plant TPKs harbor predicted 14-3-3 protein–binding domains and Ca^{2+}-binding EF-hand motifs; accordingly, their activity is

activated by coexpression of 14-3-3 proteins and elevated cytosolic Ca^{2+} levels. Recently, TPKs from *A. thaliana* (AtTPK1), barley (HvTPK1), and rice (OsTPKa) were expressed in *A. thaliana* mesophyll cell protoplasts isolated from plants lacking the two major vacuolar K^+ channels, TPK1 and TPC1. In the vacuolar membrane from these protoplasts, increased current in response to membrane tension, osmotic shock, and TNP treatment was observed (75). Although TPK channels from both plants and animals gate more readily in the presence of membrane tension, they still exhibit basal activity in the absence of tension, and are often referred to as spontaneous or leaky (e.g., 30, 39).

9. OTHER MECHANOSENSITIVE ION CHANNEL ACTIVITIES IN PLANT MEMBRANES

Although the MSLs, MCAs, and TPKs are certain to play important roles in plant biology and provide both cation- and anion-permeable MS ion channels, they are unlikely to account for all of the endogenous MS ion channel activities that have been identified in plant membranes

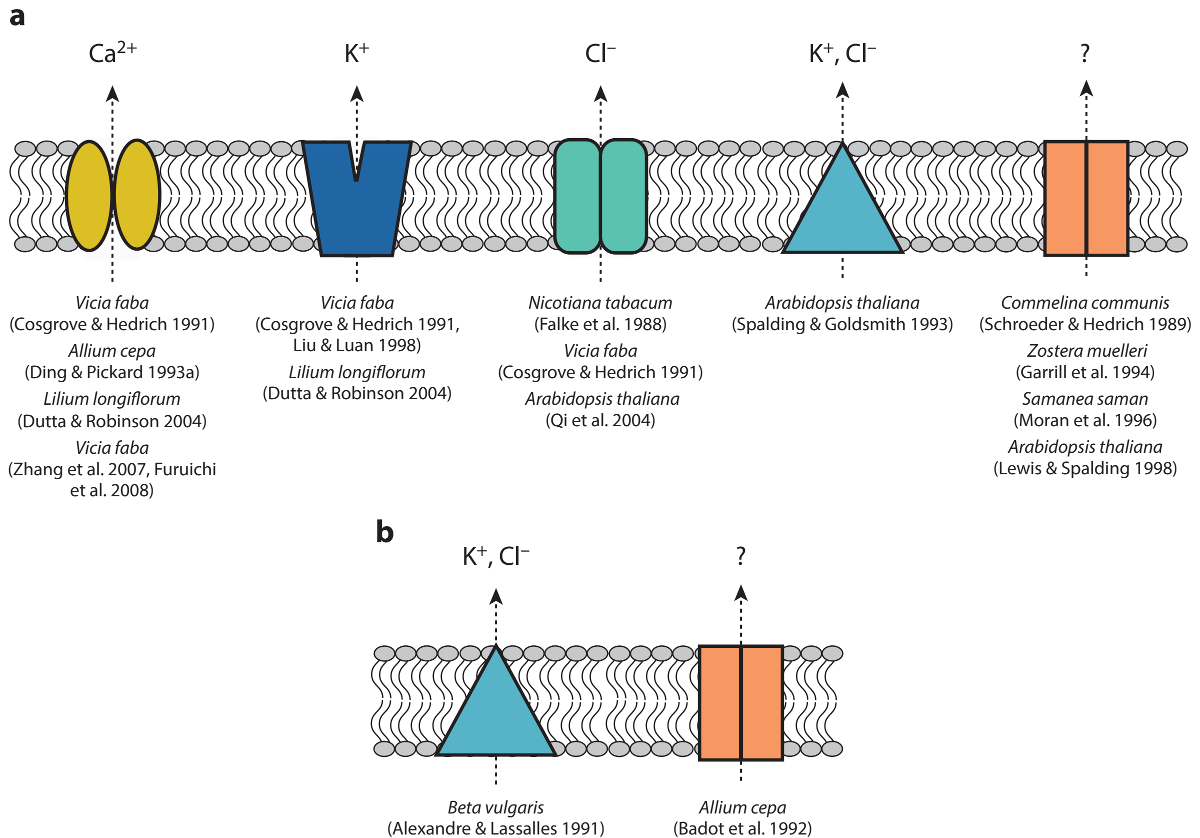


Figure 3

Molecularly uncharacterized mechanosensitive (MS) ion channel activities identified in plant membranes. Plasma membrane–localized (panel *a*) and vacuole-localized (panel *b*) MS ion channels identified through patch-clamp electrophysiology and activated through increased membrane tension are presented and categorized by established ion permeability. Relevant references are indicated beneath each channel. Arrows indicate the ion permeability but do not specify the direction of ion flux in or out of the cell or vacuole.

(**Figure 3**). Many unidentified MS ion channel activities have been observed in plant plasma membranes, including Cl^--permeable channels in *A. thaliana* mesophyll cells and stem-derived suspension cultures of *N. tabacum* (33, 104); Ca^{2+}-permeable channels in onion epidermal cells (25); and MS ion channel activities of unknown permeability in *Zostera muelleri* (38), *A. thaliana* hypocotyl cells (71), and the vacuolar membranes of onion parenchyma (5). MS ion channels permeable to both K^+ and Cl^- have been identified in the plasma membranes of *A. thaliana* mesophyll cells (111) and the vacuolar membranes of *Beta vulgaris* cells (1). Particularly interesting from a physiological standpoint may be the MS ion channel activities that have been detected in pollen grains and pollen tubes (27), guard cells (20, 37, 73, 107, 132), and the leaf-moving organ (pulvinus) of *Samanea saman* (88).

Pulvinus: an organ consisting of central vascular tissue surrounded by two groups of cortical cells whose alternating swelling and shrinking produces leaf movement

Matching each of these activities to its encoding gene will be challenging but is likely to be a rewarding endeavor. Approximately 100 MS ion channel genes have been identified in *Arabidopsis* (122). A library of these genes or cDNAs could be screened for the ability to form an MS ion channel in a variety of functional assays. Alternatively, likely candidates could be directly tested, as with two recently discovered Ca^{2+} channels that are activated by treatment with hyperosmotic solutions (50, 131).

SUMMARY POINTS

1. Mechanosensitive (MS) ion channels transduce mechanical force into biochemical signals for a wide range of physiological purposes.
2. Plants sense and respond to diverse mechanical forces, including touch, gravity, osmotic pressure, and developmental events. MS ion channels likely participate in some or all of these processes.
3. MS ion channel activities are well represented among different plant species, cell types, and cellular compartments, but only three families have been characterized in plants; other MS ion channels known to be present have not yet been identified at the molecular level.
4. A broad assortment of techniques exist to study plant MS ion channels, but additional approaches are needed to preserve the cell- and tissue-specific context in which MS ion channels function in planta.
5. A subset of MscS-like (MSL) channels localize to mitochondrial and plastidic envelopes and serve to relieve hypoosmotic stress in plastids during normal growth and development.
6. Another class of MSLs localize to the plasma membrane and endoplasmic reticulum, where they are required for the predominant MS ion channel activity in root protoplasts. The physiological functions of these channels have been elusive, although at least one has been implicated in stress-induced cell death signaling.
7. Mid1-complementing activity (MCA) proteins were identified in a functional screen in yeast. MCAs in multiple plant species are required for Ca^{2+} influx in response to mechanical events and mediate Ca^{2+} homeostasis.
8. Plant two-pore potassium (TPK) channels reside in vacuolar membranes and exhibit ion channel activity that is modulated by membrane tension.

FUTURE ISSUES

1. No plant MSL, MCA, or TPK channel has fully satisfied the four criteria for confident assignment as an MS ion channel. Establishing a physiological stimulus for MSL10 and demonstrating a change in MS ion channel properties in response to altered amino acid sequences in MSL10, MCA1, or TPK1 will be first steps toward this goal.
2. Establishing the physiological functions of plasma membrane–localized MSLs and the relevance of the structural diversity within the MSL family will require creative functional assays and new structural studies.
3. Atomic structures will be needed if we are to make significant progress in understanding the gating mechanism, regulation, and other functional aspects of plant MS ion channels. A structure will be particularly revealing for MCAs, where no information is available from homologs in other systems.
4. Current techniques for studying MS ion channels are limited in that they often require removing or damaging the cell wall and analyzing membranes outside of their natural context. New tools are needed to bypass these limitations.
5. An exciting, if ambitious, goal for the future will be to match each of the activities that have been detected in plant membranes with a known gene and corresponding channel structure. New functional screens as well as classical genetics and phylogenetics may play an important role in this endeavor.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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