United in Diversity: Mechanosensitive Ion Channels in Plants

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UNITED IN DIVERSITY: MECHANOSENSITIVE ION CHANNELS IN PLANTS

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KEYWORDS

Mechanotransduction, MscS, MSL, MCA, TPK1
ABSTRACT

Mechanosensitive (MS) ion channels are a commonly used mechanism for the perception and response 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 osmotic homeostasis of intracellular organelles. Three families of plant MS channels have been identified: the MscS-Like (MSL), Mid1-Complementing Activity (MCA), and Two-Pore Potassium (TPK) families. Channels from these three families vary widely in terms of 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 present in plant systems.
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 it 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 (102), maintaining the ionic conditions required for metabolism in plants (132) and Ca$^{2+}$ signaling in all cells (19). Interested readers are referred to two recent reviews on plant ion channel function (48, 121).

The flux of ions through a channel can be regulated by a variety of stimuli, including transmembrane voltage (10), ligand binding (57), light (23), and mechanical force (78). 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 found in all three domains of life (5, 58, 61), pointing to the fundamental requirement for mechanosensation in all cells.

Several recent reviews describe the wide variety of mechanical stimuli that land plants must sense and respond to during their lifespan (21, 85, 114). In addition, as many as 18 distinct MS ion channel activities have been identified in plant membranes by patch-clamp electrophysiology (these are described in detail below), 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 what is currently known about three families of plant MS ion channels, emphasizing their diverse structure, evolutionary history, and physiological roles.

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 (122)). The advent of the patch-clamp technique, which permitted the study of individual channels in isolated cell membranes (see Section 4.2 below), made possible the first identification of MS ion channel activities in animal skeletal cells (41, 43). Shortly thereafter, MS ion channel activities were detected in tobacco, broad bean and giant Escherichia coli protoplasts (33, 80, 106). The structures of two of the MS
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 channel activity (reviewed in (111)).

### 2.1 Models for MS Channel Gating

An ion channel can be idealized as a two-state system, where it exists in either a closed (or non-conducting) or an 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 (49).

For some classes of MS ion channels, increased membrane tension leads directly to gating. This behavior has been described by a number of biophysical models that address the energetic interactions at the membrane-protein interface (reviewed in (47)). One proposed mechanism is the lipid disordering model illustrated in Figure 1A. An ion channel increases the free energy of the membrane in which it is embedded, as the lipids in that membrane must disorder 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 (79, 115).
Another driving force for MS channel gating may be the thinning of the lipid bilayer under increased membrane tension (Figure 1B). According to this model, membrane thinning results in a mismatch between the height of the channel’s hydrophobic transmembrane (TM) domain and the profile of the lipid bilayer, leading to the exposure of nonpolar side chains to the aqueous intra- or extra-cellular 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 (77, 81). 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 (96, 98).

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 (3, 13, 47). A unifying theme in all of these models, however, is that the responsiveness of a MS ion channel to force depends on highly dynamic interactions with the lipid bilayer.
3. PHYSIOLOGICAL ROLES FOR MS CHANNELS

There are many ways in which an organism might employ a mechanosensor capable of transducing force into ion flux; many MS channels from animals have been studied in detail and shown to improve fitness during development or in a changeable environment. Here we briefly summarize what is known about the physiological roles of MS channels in *C. elegans, D. melanogaster*, and other metazoans—as these studies inform our understanding of MS channels in plants, whether or not the channels are evolutionarily related—and then address their potential functions in plants.

3.1 MS Ion Channels in Animals

Several distinct families of MS 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 *Drosophila melanogaster* (reviewed in (5)). 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 (50)). 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 (119), see sidebar: Piezo).
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, 85, 114). 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 (4, 52, 72). 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, 51, 85)).

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 degrees) is associated with membrane depolarization, the rapid influx of Ca$^{2+}$ ions and subsequent alkalination of the cells in the root cap (reviewed in (114)). Ca$^{2+}$ influx is also associated with touch stimulus (reviewed in (34)), osmotic stress (108), and bending (84), 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).

4. APPROACHES USED TO STUDY MS 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 by 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, 83, 89), ruthenium red (67) or cytoskeletal inhibitors (25). However, these treatments are often non-specific. For example, the commonly used lanthanide Gd\(^{3+}\) blocks a wide variety of channels, not only Ca\(^{2+}\)-selective or mechanosensitive (69). Furthermore, Gd\(^{3+}\) can indirectly inhibit the action of non-selective MS ion channels by reducing overall membrane fluidity (31, 74). On the other hand, the chemical trinitrophenol (TNP), which increases curvature and tension when applied to membranes (79), behaves as a MS channel activator and can induce Ca\(^{2+}\) flux or lower the threshold for mechanical stimulation (37, 89, 103). While 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 characterization of 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 the production of a high resistance seal between the glass of a micropipette tip and a small patch of membrane containing the
channels of interest (105). The pipette can either 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 channels in their native membranes or heterologously expressed in Xenopus oocytes, as described in more detail below. While 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 isolation of cells from the tissue and the removal of 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 channels in recent years. Although a forward genetic screen has not yet successfully been used to identify a MS channel, reverse genetics motivated by either phylogenetics or a functional assay has identified several candidates (45, 73, 89). Once a candidate gene is identified, the protein can be heterologously expressed and tested for MS channel activity in cell survival or electrophysiological assays. Genetic ablation or overexpression of candidate MS
channel genes *in planta* can be powerful tools for characterizing channels in their native systems.

5. CRITERIA FOR ASSIGNMENT AS A MECHANOSENSITIVE ION CHANNEL

Establishing that a particular gene encodes the primary force-transducer in a MS response, as opposed to an accessory or downstream component of the MS response, is a challenging endeavor. The following criteria have previously been established: 1) proper expression and localization for the observed MS response; 2) 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); 3) evidence of MS gating in isolation or in heterologous systems; and 4) structural alterations of the protein produce changes in the MS response and channel behavior (5, 18, 87). Assembling 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 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 channels that have so far been identified: the MscS-Like (MSL), Mid1-Complementing Activity (MCA), and Two-Pore
Potassium (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.

6. MSCS-LIKE CHANNELS

The first family of putative plant MS ion channels were identified based on their similarity to the *E. coli* Mechano-sensitive channel of Small conductance (MscS), a well-established model system for the study of MS ion channels ([12, 82], see sidebar: MscS). MscS serves as an “emergency release valve” under conditions of hypoosmotic shock in *E. coli* ([12, 68]). 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 ([7, 44, 59, 60, 76, 100, 101, 126]). MscS homologs have not yet been identified in animal genomes.

The predicted evolutionary relationship among representative members of the MscS superfamily is presented in Figure 2A, using the ~100 amino acid domain conserved among the MscS superfamily (99). This sequence maps to the pore-lining helix and the upper part of the cytoplasmic vestibule of MscS and is marked in cyan in Figure 2B (8). A number of highly conserved motifs within this domain are important for function in bacterial and plant channels ([7, 22, 53]). Land plant MscS homologs fall into three
phylogenetic groups, I-III, which also correspond to three different subcellular localizations (see below).

6.1 A Diverse Family of MS 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 found appended to the basic MscS topology (76, 116, 126). 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 2B).

Group I and Group II MSL proteins are predicted (and in some cases, have been shown) to localize to mitochondria and to plastids, respectively (44, 45). 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 shown to localize to the plasma membrane (44, 46) 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 four and five, 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 (44). 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 (44). 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).

### 6.2 Evidence that MSLs are MS Ion Channels

It is currently accepted that MS ion channel activity has been retained among most members of the MscS superfamily (however, for one exception see (15)). All six MscS family members in *E. coli* are capable of producing tension-gated activities in giant spheroplasts (28, 68, 70, 107), as is MSC1 from *Chlamydomonas reinhardtii*, (91), and MSY1 from fission yeast (92).

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 of an *E. coli* strain missing three major MS ion channels to hypoosmotic shock (45). More direct evidence was obtained for the endoplasmic reticulum (ER)- 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 (46). A characterization of the conductance of MS ion channel activities present in root protoplasts from single ms9, single ms10 or double ms9 ms10 mutants suggests that MS9 and MS10 can form a heteromeric channel with a conductance of ~50 pS, while MS9 and MS10 homomeric channels have conductances of ~45 pS and ~140 pS, respectively (46, 97). 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 MS10 is associated with a ~100 pS MS ion channel activity when expressed heterologously in Xenopus oocytes (75). MS10 channel activity in oocytes has a slight (6-fold) preference for anions, and closes at lower tensions than it opens. MS10 meets three of the four criteria for a bona fide MS ion channel: 1) MSL10 is expressed in root cells, where 2) it is required for the wild type MS ion currents but not for the normal development of the tissue, and 3) expression of MSL10 in Xenopus oocytes confirms 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 (but distinct from) function as an emergency release valve. 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 (45, 125). Immunofluorescence of algal MSC1 similarly revealed a complex localization pattern of punctate spots both within the chloroplast and the cytoplasm (91).

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 non-green plastids in the epidermis and root (small, ovoid plastids are seen in wild type epidermal cells) (45). These defects in plastid size and shape can be rescued by increasing the osmolarity of the cytoplasm relative to the plastid by a variety of genetic and environmental manipulations (117), strongly suggesting that non-green 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* mutants have fewer and larger chloroplasts than the wild type, possibly as a result of the multiple FtsZ rings observed in the chloroplasts of this mutant (125). As MSL2 and MSL3-GFP fusion proteins co-localize with the
plastid division protein AtMinE (45), it is possible that MSL2 and MSL3 interact with the plastid division machinery to influence division site selection; it is equally feasible 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 (124). A function for MS channels in division is evolutionarily conserved, as E. coli mutants lacking several key MS channels also exhibit defects in division site selection when exposed to the division inhibitor cephalexin (125). 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 (91).

Single msl2 and double msl2 msl3 mutants also exhibit a number of whole-plant phenotypes, including dwarfing, rumpled leaf surfaces, thicker leaf lamina, and variegation (45, 53, 125). While 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 (123). MSL2 and MSL3 appear to function partially redundantly to relieve plastid osmotic stress. While a null msl2 allele produces developmental defects even in the wild type MSL3 background (53), all mutant phenotypes are exacerbated in the msl2 msl3 double mutant (45, 117, 123, 125). There is currently no null msl3 allele, and it remains to be established exactly how the functions of MSL2 and MSL3 overlap and diverge.
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 a msl4 msl5 msl6 msl9 msl10 quintuple null mutant (46, 114). This may be surprising, given that MSY1 and MSY2, which localize to the ER of Schizosaccharomyces pombe, play an essential role in protecting cells from hypoosmotic shock (92). 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 leads to dwarfing, H$_2$O$_2$-associated cell death, and the induction of cell death-associated gene expression (116).

6.5 Beyond the Paradigm of Emergency Release Valves

While 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 and complex roles in both prokaryotes and eukaryotes (reviewed in (11, 22, 76, 126)). Based on the diverse localization, topology, and domain structure within the MscS superfamily, we have previously suggested that 1) some MscS-like channels may respond to osmotic stress other than that provided by the extracellular environment, 2) some may be regulated by
mechanisms other than membrane tension, and 3) some might even have functions that are completely separable from their role in mediating ion flux (47).  

Experimental support for these three ideas in A. thaliana MSLs has accumulated over the past decade. For example, 1) 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 MscS-like channels can serve to protect organellar membranes this stress during normal growth and development (45, 117). Furthermore, 2) 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 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 (116), and MSL9 is a direct target of the drought-associated kinase SnRK2.6 (120). Finally, 3) 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 (116). 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.
7. MID1-COMPLEMENTING ACTIVITY CHANNELS

While MSLs are essential to organelle osmoregulation and likely play complex roles at the plasma membrane and ER, they are essentially non-selective 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 the novel land plant-specific family of membrane-associated proteins called **Mid1-Complementing Activity (MCA)**. Only one or two family members are found in each plant genome, and homologs have not been found in algae or animals (64). Sequence conservation is not restricted to a single domain, but 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 family, *A. thaliana* MCA1, was identified in a functional screen for cDNAs capable of rescuing the *mid1* mutant strain of *Saccharomyces cerevisiae* (89). Mid1 is a stretch-activated MS ion channel required for Ca\(^{2+}\) influx and cell survival after exposure to mating pheromone (56). *A. thaliana* MCA2 and *Nicotiana tabacum* MCA1 and MCA2 were identified based on homology to AtMCA1 and are also capable of promoting the survival of *mid1* yeast in the mating factor assay (64, 129).

MCA proteins from *A. thaliana*, rice, and tobacco 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 (63, 64, 89, 129). 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 a MS calcium channel.

Most MCA-GFP fusion proteins localize to the plasma membrane of plant cells, often in a punctate pattern (63, 89, 90, 129). When expressed in yeast, MCA1 fractionates with plasma membrane proteins and behaves like an intrinsic membrane protein in solubility tests (89, 90). The overexpression of MCA proteins is associated with increased influx of Ca$^{2+}$ into plant roots, plant tissue culture cells, 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 (63, 64, 89, 129). 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, 64, 89, 95).

MCA genes are expressed broadly in a variety of tissues (63, 89, 129) and MCA T-DNA insertion mutants in A. thaliana and OsMCA1-silenced lines in rice show growth defects and late flowering (63, 129). AtMCA1 and AtMCA2 have partially divergent functions; mca1 but not mca2 mutants show defects in root entry into hard agar (89, 129), while mca2 but not mca1 mutants are defective in Ca$^{2+}$ uptake in A. thaliana roots (129). The mca1 mca2 double mutant exhibits both of these phenotypes, as well as growth that is
hypersensitive to Mg\(^{2+}\) (probably due to competition with Ca\(^{2+}\) for uptake) (129). Further evidence that MCAs are involved in signaling in response to membrane tension comes from studies on the cellular response to treatment with the cell wall biosynthesis inhibitor isoxaben, which leads to cellular swelling (66). 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, 42, 127).

### 7.2 Structural Features of MCAs

Surprisingly, MCAs do not resemble 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 (62). The membrane-spanning domains and topology of MCAs is 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) (90). Gel migration, gel filtration and crosslinking studies indicate that MCA1 and MCA2 form homotetramers (90, 109). 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 (109).
Like MSL10, MCA-associated channel activity has been characterized in Xenopus oocytes. Using 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 to 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 falls short of establishing this unequivocally by introducing a mutation that alters channel behavior. As the single channel activities attributed to MCA1 appear 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 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, voltage-independent and can often be activated by increased \([\text{Ca}^{2+}]_{\text{cyt}}\) (30). Membrane tension has been shown to modulate the open probability of several mammalian TPKs (9, 17) and they are proposed to play a variety...
of mechanosensory roles in cardiomyocytes, the smooth muscle of the stomach and intestines, and in pain perception (50).

In plants, a subset of TPK channels is localized to the vacuolar membrane (summarized in (118)). 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 co-expression 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 (73). While 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 MS CHANNEL ACTIVITIES IN PLANT MEMBRANES

While the MSLs, MCAs and TPKs are certain to play important roles in plant biology, and provide both cation- and anion-permeable MS channels, they are unlikely to
account for all of the endogenous MS ion channel activities that have been identified in plant membranes (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, 103); 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 (69), and the vacuolar membranes of onion parenchyma (6). MS ion channels permeable to both K⁺ and Cl⁻ have been identified in the plasma membrane of A. thaliana mesophyll cells (110) and in the vacuolar membranes of Beta vulgaris (2). Particularly interesting from a physiological standpoint may be the MS ion channel activities that have been detected in pollen grains and pollen tubes (27), in guard cells (20, 37, 71, 106, 130), and in the leaf-moving organ (pulvinus) of Samanea saman (86).
1. 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 are likely participants 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 yet 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 channel function in planta.

5. A subset of 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 ER where they are required for the predominant MS channel activity in root protoplasts. The physiological function(s) of these channels have been elusive, though at least one has been implicated in stress-induced cell death signaling.
7. 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 TPK channels reside in vacuolar membranes and exhibit ion channel activity that is modulated by membrane tension.
FUTURE ISSUES LIST

1. No plant MSL, MCA, or TPK channel has fully satisfied the four criteria for confident assignment as a MS channel. Establishing a physiological stimulus for MSL10 and demonstrating a change in MS channel properties in response to mutations in MCA1, MSL10, or TPK1 will be first steps towards 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 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 removal or damage of 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 forward genetics and phylogenetics may play an important role in this endeavor.
ACKNOWLEDGEMENTS

We thank our many colleagues in the plant biology, mechanobiology, and signal transduction communities, and apologize to those whose work we were unable to include due to size constraints. We are grateful to the current members of the Haswell lab for insightful comments on this manuscript while it was in preparation. Our current work on MS ion channels in plants is supported by NSF (MCB-1253103), NASA (NNX13AM55G) and NIH (2R01GM084211).


64. Kurusu T, Yamanaka T, Nakano M, Takiguchi A, Ogasawara Y, et al. 2012. Involvement of the putative Ca^{2+}-permeable mechanosensitive channels, NtMCA1 and NtMCA2, in Ca^{2+} uptake, Ca^{2+}-dependent cell proliferation and mechanical


1. **Ion channel** = A gated macromolecular pore in a cell membrane that, once opened, permits ions to flow down their electrochemical gradient.

2. **Gating** = Conformational change undergone by an ion channel in response to stimuli that creates an ion-permeable pore through the membrane.

3. **Conductance** = A measurement of the ease with which current flows through an ion channel at a given voltage, measured in Siemens (S).

4. **Open probability** = the ratio of channels that are open to those that are closed in a particular population.

5. **Patch-clamp electrophysiology** = A technique for measuring current across an isolated patch of membrane under conditions that maintain a particular transmembrane voltage.

6. **MS** = mechanosensitive

7. **Protoplast** = a bacterial, fungal, or plant cell from which the cell wall has been removed.

8. **Hypoosmotic shock** = Rapidly decreasing the osmolarity of the media for a membrane-bound cell or organelle; results in water influx into the cell.

9. **MSL** = MscS-Like; MS channels that protect cells/organelles from osmotic stress in bacteria, archaea, fungi, and plants; may have additional physiological functions.
10. MCA = Mid1-Complementing Activity; plasma membrane-localized MS calcium channels that mediate osmotic stress response and calcium homeostasis in plants.

11. TPK = Two-pore Potassium (K⁺): mechanically modulated, potassium-selective channels that localize to the plant vacuole and participate in ion homeostasis.

12. Plastid = A plant-specific endosymbiotic organelle in which photosynthesis, biosynthesis and/or storage of cell metabolites take place.


14. TNP = Trinitrophenol or picric acid; a negatively charged amphipath that inserts into the outer bilayer of a membrane and induces curvature.

15. CHO cells = Chinese Hamster Ovary cells, a commonly used cell line for protein expression and tissue culture work.

16. Isoxaben = Herbicide that prevents the incorporation of glucose into cell walls by inhibiting cellulose synthase subunits.

17. DEG/ENaC = Degenerin/Epithelial sodium (Na) Channels; cation-selective ion channels that mediate touch response in animals; especially well-characterized in C. elegans.

18. TRP = Transient Receptor Potential channels; a family of cation-selective candidate mechanosensitive ion channels potentially mediating multiple sensory pathways in animals.

19. FtsZ = Filamentous temperature sensitive Z; a GTPase that forms filaments required for fission in bacteria and plastids.
20. Pulvinus = Organ consisting of central vascular tissue surrounded by two groups of cortical cells whose alternate swelling/shrinking produces leaf movement.
Sidebar1: Piezo channels (line 162)

Piezo channels, named for the Greek word for pressure, are believed to mediate the perception of mechanical stimuli in animal systems (reviewed in (94, 119, 128)). 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 were identified throughout the animal kingdom, in protists, amoebae, and, surprisingly, in plants. Piezos are exceptionally large proteins, comprising 2000-4000 amino acids and 20-40 predicted transmembrane helices. Expressed in both sensory and non-sensory tissues, Piezos are required for response to gentle touch and for vascular development in Zebrafish and mouse. They are further implicated in noxious touch response, cellular extrusion, and red blood cell volume regulation in flies, fish, mouse, rat, and humans. While heterologous expression of Piezo channels can confer mechanosensitivity on an insensitive cell, it is not yet known if they require other cellular components or a specialized lipid environment for mechanosensitivity. Mutations in human Piezo genes are associated with a number of diseases.

Sidebar2: Escherichia coli MscS (line 259)

The Mechanosensitive channel of Small conductance 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 (12, 47, 61)). MscS is a weakly anion-preferring channel with a conductance of ~1 nS, and contributes to cellular survival of hypoosmotic shock ranging
from 500-1000 mOsm. 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 osmolytes that are available to pass through the channel pore. Five crystal structures of prokaryotic MscS homologs in conducting and non-conducting 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.
Figure 1. Models for Mechanosensitive Ion Channel Gating. In the lipid reordering model (a) MS ion channels force the membrane to distort to establish favorable interactions with the channel (top). Lateral membrane tension (horizontal arrows) increases bilayer energy as the membrane structure is further altered. The open conformation of the channel is then favored as 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 channel relative to the membrane emphasized by dashed lines. In the hydrophobic mismatch model (b), membrane bilayers create favorable interactions between the polar lipid heads and polar residues of an embedded protein (top). Lateral membrane tension (horizontal 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). Increasing hydrophobicity of regions is shown as a gradient from very hydrophilic (red) to very hydrophobic (blue).

Figure 2 Phylogenetic Relationships and Subcellular Localization, and Topologies of MscS-Like Channels. (a). The inferred phylogeny of 44 members of the MscS superfamily is presented as an unrooted radial tree. Sequences were identified by Phytozome BLAST analysis (http://www.phytozome.net/) or inclusion in previous analyses (15, 44, 65, 92, 93, 100, 117, 131). The MscS-like region of each protein was
identified by InterProScan (55) and aligned using ClustalW (113) 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 (104) method with a JTT distance matrix (54) using MEGA6 software (112). 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. Scale bar, 4.0 amino acid substitutions per site. The phylogenetic origin or cluster is indicated in the colored boxes. The sequences used in this analysis and their UniProt accession numbers, TAIR accession numbers, or Phytozome (cite) accession numbers are: *E. coli* MscS (P0C0S1), YbdG (P0AAT4); *Synechocystis* sp. PCC6803 bCNAGa (M1ME31); *H. pylori* MscS (E1Q2W1); *C. glutamicum* MscCG (P42531); *T. tengcongensis* MscS (Q8R6L9); *T. gondii* (B6KM08); *P. falciparum* (Q8lIS3); *D. discoideum* (Q54ZV3); *S. pombe* MSY1 (O74839), MSY2 (O14050); *C. reinhardtii* MSC1 (A3KE12), MSC2 (A8HM43), MSC3 (A8HM47); *A. thaliana* MSL1 (At4g00290), MSL2 (At5g10490), MSL3 (At1g58200), MSL8 (At2g17010), MSL9 (At5g19520), MSL10 (At5g12080); *P. trichocarpa* (Pt002G105900, Pt004G178900); *Z. mays* (GRMZM2G125494, GRMZM2G028914, GRMZM2G005013); *O. sativa* (Os02g45690, Os04g48940, Os06g10410, Os02g44770); *B. distachyon* (Bradi1g15920, Bradi5g19160, Bradi3g51250); *V. vinifera* (Vv00015105001, Vv00026926001, Vv00002410001); *P. patens* (Pp1s79_156, Pp1s314_12, Pp1s2_4320); *C. papaya* (supercontig_55.26, _22.80, _126.38_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/index.ep). The
region of highest homology to *E. coli* MscS is cyan, the Group I-specific C-terminal extension is green, and the Group III-specific N-terminal region is highlighted in orange.

**Figure 3. Molecularly Uncharacterized MS Ion Channel Activities Identified in Plant Membranes.** (a) Plasma membrane- and (b) vacuolar-localized MS ion channels identified through patch-clamp electrophysiology and activated through increased membrane tension are presented and categorized by established ion permeability. Relevant citations 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.
Table 1. Plant Mechanosensitive Ion Channels

<table>
<thead>
<tr>
<th>Family</th>
<th>Protein</th>
<th>Gene</th>
<th>Organism</th>
<th>Mutant/Silenced Phenotype</th>
<th>Overexpression phenotype</th>
<th>Subcellular Localization</th>
<th>Associated MS Ion Channel Characteristics</th>
<th>Activity in Heterologous Systems</th>
<th>Key References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mid-1 Complementing Activity</strong></td>
<td>MCA1</td>
<td>At4g35520</td>
<td>Arabidopsis thaliana</td>
<td>the roots of mca2 mutants are less efficient at generating hard agar, do not induce lignin deposition upon wounding, glucose biosynthesis, and starch accumulation</td>
<td>increased Ca⁺⁺-uptake in seedling roots, increased Ca⁺⁺-influx (measured as aequorin signal) in response to hypoosmotic shock and TNF treatment, high level expression of TCH3</td>
<td>plasma membrane</td>
<td>expression is associated with ~15 or ~35 pS conductance</td>
<td>survival and Ca⁺⁺-uptake in response to hypoosmotic shock and TNF treatment, high level expression of TCH3</td>
<td>Nakagawa et al., 2007; Yamazaki et al., 2010; Boscari et al., 2011; Morrell et al., 2012; Furuchi et al., 2012.</td>
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<tr>
<td></td>
<td>MCA2</td>
<td>A2g17780</td>
<td>Arabidopsis thaliana</td>
<td>mca2 mutants show a reduction in Ca⁺⁺-uptake, mca7 mca2 double mutants are hypersensitive to MgCl₂ and show developmental delay.</td>
<td>plasma membrane</td>
<td>survival and Ca⁺⁺-uptake in response to hypoosmotic shock</td>
<td>Yamasaki et al., 2010</td>
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<tr>
<td></td>
<td>NIMCA1</td>
<td>At5g2811</td>
<td>Nicotiana tabacum</td>
<td>increased Ca⁺⁺-uptake in cultured tobacco cells, higher expression of MIERF4</td>
<td>plasma membrane, punctate signal</td>
<td>survival and Ca⁺⁺-uptake in response to hypoosmotic shock in S. cerevisiae</td>
<td>Kusuru et al., 2011</td>
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<tr>
<td></td>
<td>NIMCA2</td>
<td>At5g2812</td>
<td>Nicotiana tabacum</td>
<td>increased Ca⁺⁺-uptake in cultured tobacco cells, higher expression of MIERF4</td>
<td>plasma membrane, punctate signal</td>
<td>survival and Ca⁺⁺-uptake in response to hypoosmotic shock in S. cerevisiae</td>
<td>Kusuru et al., 2011</td>
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<tr>
<td></td>
<td>OsMCA1</td>
<td>O03g0157300</td>
<td>Oryza sativa</td>
<td>OsMCA1 silenced lines exhibit slower growth, reduced aquaporin transcript levels, and increased Ca⁺⁺-influx in response to hypoosmotic shock</td>
<td>increased Ca⁺⁺-uptake in cultured rice cells</td>
<td>plasma membrane</td>
<td></td>
<td>Kusuru et al., 2012</td>
<td></td>
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<tr>
<td><strong>MacS-Like</strong></td>
<td>MSL1</td>
<td>At4g00200</td>
<td>Arabidopsis thaliana</td>
<td>mca2 null mutants show defective leaf shape, mca7 mca2 double mutants have enlarged chloroplasts and enlarged, round non-green plastids; mca2 mca7 double mutant chloroplasts exhibit multiple division rings.</td>
<td>mAChR mRNA expression is associated with ~15 or ~35 pS conductance</td>
<td>mitochondrial</td>
<td>survival of hypo-osmotic shock in E. coli</td>
<td>Haswell, 2007</td>
<td></td>
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<tr>
<td></td>
<td>MSL2</td>
<td>A1g10490</td>
<td>Arabidopsis thaliana</td>
<td>mca2 null mutants show defective leaf shape, mca7 mca2 double mutants have enlarged chloroplasts and enlarged, round non-green plastids; mca2 mca7 double mutant chloroplasts exhibit multiple division rings.</td>
<td>mAChR mRNA expression is associated with ~15 or ~35 pS conductance</td>
<td>mitochondrial</td>
<td>survival of hypo-osmotic shock in E. coli</td>
<td>Haswell, 2007; Wilson et al., 2011; Jensen &amp; Haswell, 2011; Veley et al., 2012</td>
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<tr>
<td></td>
<td>MSL3</td>
<td>A1g58200</td>
<td>Arabidopsis thaliana</td>
<td>mca2 double mutants have enlarged chloroplasts and enlarged, round non-green plastids; mca2 mca7 double mutant chloroplasts exhibit multiple division rings.</td>
<td>mAChR mRNA expression is associated with ~15 or ~35 pS conductance</td>
<td>mitochondrial</td>
<td>survival of hypo-osmotic shock in E. coli</td>
<td>Haswell, 2007; Wilson et al., 2011; Jensen &amp; Haswell, 2011; Veley et al., 2012</td>
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<td></td>
<td>MSL4</td>
<td>A1g33470</td>
<td>Arabidopsis thaliana</td>
<td>mca2 mca7 mca8 mca9 mutants lack MS channel activity in root protoplasts</td>
<td>mAChR mRNA expression is associated with ~15 or ~35 pS conductance</td>
<td>plasma membrane</td>
<td>survival of hypo-osmotic shock in E. coli</td>
<td>Haswell, Paynemiat et al., 2008</td>
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<td></td>
<td>MSL5</td>
<td>A1g14810</td>
<td>Arabidopsis thaliana</td>
<td>mca2 mca7 mca8 mca9 mutants lack MS channel activity in root protoplasts</td>
<td>mAChR mRNA expression is associated with ~15 or ~35 pS conductance</td>
<td>plasma membrane</td>
<td>survival of hypo-osmotic shock in E. coli</td>
<td>Haswell, Paynemiat et al., 2008</td>
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<td></td>
<td>MSL6</td>
<td>A1g78610</td>
<td>Arabidopsis thaliana</td>
<td>mca2 mca7 mca8 mca9 mutants lack MS channel activity in root protoplasts</td>
<td>mAChR mRNA expression is associated with ~15 or ~35 pS conductance</td>
<td>plasma membrane</td>
<td>survival of hypo-osmotic shock in E. coli</td>
<td>Haswell, Paynemiat et al., 2008</td>
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<td></td>
<td>MSL9</td>
<td>A1g19520</td>
<td>Arabidopsis thaliana</td>
<td>mca2 mca7 mca8 mca9 mutants lack MS channel activity in root protoplasts</td>
<td>mAChR mRNA expression is associated with ~15 or ~35 pS conductance</td>
<td>plasma membrane</td>
<td>survival of hypo-osmotic shock in E. coli</td>
<td>Haswell, Paynemiat et al., 2008</td>
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<tr>
<td></td>
<td>MSL10</td>
<td>A1g12080</td>
<td>Arabidopsis thaliana</td>
<td>mca2 mca7 mca8 mca9 mutants lack MS channel activity in root protoplasts</td>
<td>mAChR mRNA expression is associated with ~15 or ~35 pS conductance</td>
<td>plasma membrane</td>
<td>survival of hypo-osmotic shock in E. coli</td>
<td>Haswell, Paynemiat et al., 2008; Maksaev and Haswell, 2012; Veley et al., 2014</td>
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<td><strong>Two-Pore K⁺</strong></td>
<td>TPK1</td>
<td>At4g56630</td>
<td>Arabidopsis thaliana</td>
<td>MTK mutants lack an instantaneous tonoplast K⁺-current</td>
<td>mAChR mRNA expression is associated with ~15 or ~35 pS conductance</td>
<td>plasma membrane</td>
<td>survival of hypo-osmotic shock in E. coli</td>
<td>Haswell, Paynemiat et al., 2008; Maksaev and Haswell, 2012; Veley et al., 2014</td>
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<tr>
<td></td>
<td>OsTPK1a</td>
<td>O03p341002</td>
<td>Oryza sativa</td>
<td>mAChR mRNA expression is associated with ~15 or ~35 pS conductance</td>
<td>plasma membrane</td>
<td>survival of hypo-osmotic shock in E. coli</td>
<td>Haswell, Paynemiat et al., 2008; Maksaev and Haswell, 2012; Veley et al., 2014</td>
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<tr>
<td></td>
<td>HvTPK3</td>
<td>EU926490</td>
<td>Hordeum vulgare</td>
<td>mAChR mRNA expression is associated with ~15 or ~35 pS conductance</td>
<td>plasma membrane</td>
<td>survival of hypo-osmotic shock in E. coli</td>
<td>Haswell, Paynemiat et al., 2008; Maksaev and Haswell, 2012; Veley et al., 2014</td>
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* predicted