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Plant mechanosensitive ion channels: an ocean of possibilities

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Mechanosensitive ion channels, transmembrane proteins that directly couple mechanical stimuli to ion flux, serve to sense and respond to changes in membrane tension in all branches of life. In plants, mechanosensitive channels have been implicated in the perception of important mechanical stimuli such as osmotic pressure, touch, gravity, and pathogenic invasion. Indeed, three established families of plant mechanosensitive ion channels play roles in cell and organelle osmoregulation and root mechanosensing — and it is likely that many other channels and functions await discovery. Inspired by recent discoveries in bacterial and animal systems, we are beginning to establish the conserved and the unique ways in which mechanosensitive channels function in plants.

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Introduction

The ability to sense intrinsic or extrinsic mechanical cues is as basal to the tree of life as the ownership of a cell membrane [1•]. Several aspects of growth and development in land plants involve mechanical signals, including touch, osmotic stress, vibration, and gravity responses, the perception of pathogen invasion, and proprioception. Well-established components of the mechanosensory apparatus of cells in every kingdom are mechanosensitive (also called stretch-activated) (MS) ion channels [2–4]. These multimeric pore-forming proteins convert mechanical force into ion flux. In some cases, the flow of ions through an open MS ion channel is sufficient for the desired response to mechanical stimulation. For example, the canonical bacterial MS ion channel MscS acts as an osmotic safety valve to protect the cell from hypo-osmotic stress; passage of ions out of the cell through channel directly accomplishes the primary function of the channel [5]. In other cases, mechanosensitive ion flux generates bioelectric signals that in turn trigger organismal sensory perception. For example, the MS ion channel NOMPC mediates touch perception in *Drosophila* larvae [6]. The distinction between the two examples above may not be so clear, as a recent report demonstrated entry of the second messenger Ca^{2+} into the bacterial cell through MscS during hypoosmotic shock [7]. In this article, we summarize recent exciting developments in the field of plant MS channels, speculate on their evolution, describe a few areas of limited knowledge, and propose potential solutions to technical challenges.

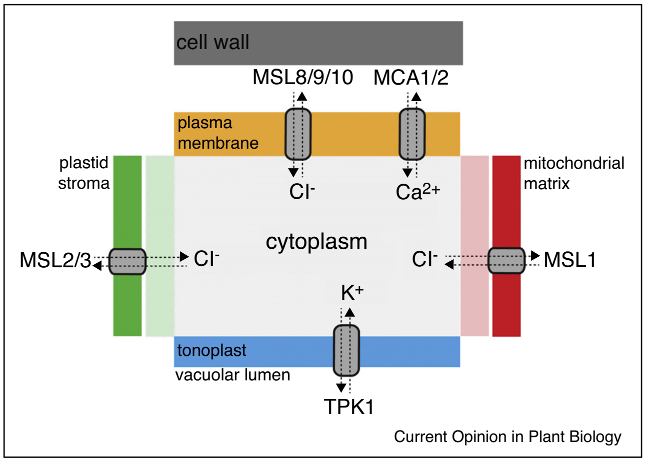
The tip of the iceberg: known families of plant mechanosensitive channels

The first MS channel activities in plant membranes were characterized by patch clamp electrophysiology [8,9] shortly after they were discovered in animal cells (see [10] for a historical perspective). Dozens of MS channel activities in the plasma and vacuolar membranes of a wide variety of cell types and species have been described over the past 30 years (summarized in [11]), suggesting that they are used broadly in plants to respond to diverse signals. Despite this apparent ubiquity, the underlying genes/proteins and physiological function of only a handful of MS ion channel activities have been elucidated. So far, three MS channel families have so far been characterized as membrane stretch-activated in plant systems; as described in further detail below, these channels exhibit diverse yet overlapping localization, structure, channel properties and proposed function. As a result, the activity of channels with different ionic affinities in the same or in different compartments is likely to have complex effects on ion flux into and out of the cytoplasm and apoplast (Figure 1). These three families are unlikely to provide all observed MS channel activities in plants, and a major challenge for the field will be the development of functional (rather that homology-based) screens capable of identifying additional MS channels. Intriguing candidates have been identified [12–14] but have not yet been shown to respond directly to membrane tension.

MscS-like (MSL) channels

Escherichia coli MscS is one of the best-understood MS ion channels in any system. It is an essentially non-selective ion channel, gated directly by membrane tension, with a large conductance of 1.2 nS. The classic function of *Ec*MscS is to serve as an osmotic safety valve, protecting cells from rupture during extreme hypo-osmotic downshock. MscS-Like channels, or MSLs, are found throughout bacteria, archaea, some fungi, algae, and plants [15]. *MSL* gene families have been described and characterized to various

Figure 1



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Subcellular localization and ionic preference for known plant mechanosensitive ion channels. The subcellular localization of MS ion channel proteins identified to date in land plants is indicated [20,21,22,23••,32••,58]. The outer membrane of the chloroplast is permeable to ions [59], and Voltage-dependent Anion Channels (VDACs) are thought to mediate flux across the outer mitochondrial membrane [60]. MSL, MscS-Like; TPK, Two-pore K^+; MCA, Mid1-Complementing Activity. Note that only general ion permeability preferences are indicated; these channels are likely to be permeable to additional species.

degrees in Arabidopsis, papaya, rice, and common bean [16–19]. There are 10 MSL proteins in Arabidopsis, most of which are predicted to localize to the plasma membrane. Unexpectedly, MSL1, MSL2, and MSL3 were found to localize to the inner membrane of plastids and mitochondria (Figure 1 [20–22,23••]).

Electrophysiological analyses of MSL9 and MSL10 in plant cells [22], MSL10 and MSL8 expressed heterologously in Xenopus oocytes [23••,24•], and MSL1 expressed heterologously in giant *E. coli* spheroplasts [21] all revealed channel characteristics that are similar (though not identical) to *Ec*MscS. MSLs are anion-preferring (e.g. 2–6 anions pass for every cation) MS ion channels with conductances ranging from ~0.1 to 1 nS depending on buffer conditions. Several lines of evidence support the model that, like *Ec*MscS, *At*MSLs function to relieve osmotic stress. This was first demonstrated with MSL2 and MSL3, two plastid-localized channels that directly maintain plastid osmoregulation. Plastids in *msl2 msl3* mutants exhibit altered size, shape and fission [20,25,26]. The loss of MSL2/3 also leads to stress responses associated with drought and the development of callus tissue at the apex of the plant [27,28]. While the pleiotropic phenotypes associated with this mutant have illustrated the importance of plastid osmoregulation during normal plant growth and development, any mechanistic insights await the electrophysiological analysis of MSL2 and MSL3 — a challenging prospect for plastid-localized proteins. Adding to the complexity is a recent report demonstrating that mitochondria-localized MSL1 is required to ameliorate the oxidative burden imposed upon mitochondria during abiotic stress [21]. The potential roles of membrane tension, redox state, and transmembrane voltage in regulating MSL1 channel activity *in vivo* remain to be determined. For plasma membrane-localized MSLs, recent reports both support their role as osmotic safety valves and suggest more complex function, as discussed below.

Two-pore domain K^+ (TPK) channels

TREK1, TREK2, and TRAAK are MS channels from the TPK family expressed in the mammalian nervous system and proposed to modulate mechanical-, heat- and cold-associated pain perception [29]. *At*TPK1 is a voltage-independent K^+ channel required for normal guard cell closure kinetics [30], and, along with homologs from rice and barley, has been demonstrated to be mechanosensitive [31]. Whether the mechanosensitive activity of *At*TPK1 is important for its function in guard cells, and how it might be integrated with other regulatory signals such as low pH, Ca^{2+} and binding to 14-3-3 proteins is not yet understood [30].

Mid1-Complementing Activity (MCA) channels

The Mid1-Complementing Activity (MCA) proteins were identified based on their ability to rescue the mating-induced lethality of the yeast *mid1* mutant [32••]. MCA proteins are plant-specific and show no homology to the yeast Mid1 channel. In fact, MCA proteins have only 1 transmembrane (TM) domain [33], placing them outside the norm for ion channel subunits. Cryo-EM imaging followed by single particle reconstruction of a MCA2 tetramer did not reveal a pore [34]. However, heterologously expressed MCA1 and 2 produce increased current in response to osmotic swelling in whole cells and to membrane stretch in excised patches [35], providing evidence that they directly form a MS ion channel. *MCA* expression is correlated with enhanced Ca^{2+} influx in response to hypoosmotic shock and mechanical stimulus in several plant species [32••,36,37]. Arabidopsis *MCAs* are required for normal rates of root penetration into hard agar and for proper response to cellulose biosynthesis inhibition, implying a role in the maintenance/response to extracellular mechanical stress [32••,38]. MCAs may be involved in the perception of developmentally imposed mechanical signals, as a maize MCA homolog was recently identified in a screen for leaf patterning mutants [39].

Getting our sea legs: recent advances in understanding plasma membrane localized MSL channels

MSL8 fully meets the criteria for a mechanoreceptor

A recent analysis of MSL8, a MS ion channel expressed exclusively in mature pollen grains and tubes, advances our understanding of the function of plasma membrane-localized MSL channels and underscores the essential role