

1-27-2015

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Grigory Maksaev

Washington University in St Louis

Kira M. Veley

Washington University in St Louis

Elizabeth S. Haswell

Washington University in St Louis, ehaswell@wustl.edu

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Recommended Citation

Maksaev, Grigory; Veley, Kira M.; and Haswell, Elizabeth S., "A Structure-Function Approach to Understanding the Dual Functions of the Plant Mechanosensitive Ion Channel MSL10" (2015). *Biology Faculty Publications & Presentations*. 106.

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A Structure-Function Approach to Understanding the Dual Functions of the Plant Mechanosensitive Ion Channel MSL10

Grigory Maksaev, Kira Veley, Elizabeth Haswell

Biophysical Society, 59th annual meeting, Feb. 7-11, 2015, Baltimore

In Brief: MscS-Like (MSL) 10 is a member of the MscS superfamily of mechanosensitive ion channels and one of 10 MSL proteins in the model flowering plant *Arabidopsis thaliana*. Unlike *Escherichia coli* MscS, MSL10 contains 6 transmembrane helices, and only its C-terminal TM helix shows homology with EcMscS's pore-lining domain. MSL10 has been shown to provide a mechanosensitive activity in plant cells and *Xenopus* oocytes. However, its structural organization and function in plants are just beginning to be elucidated.

MscS-Like (MSL) 10 is a member of the MscS superfamily of mechanosensitive ion channels and one of 10 MSL proteins in the model flowering plant *Arabidopsis thaliana*. Unlike *Escherichia coli* MscS, MSL10 contains 6 transmembrane helices, and only its C-terminal TM helix shows homology with EcMscS's pore-lining domain. MSL10 has been shown to provide a mechanosensitive activity in plant cells and *Xenopus* oocytes. However, its structural organization and function in plants are just beginning to be elucidated. We have shown that MSL10 is involved in one or more signal pathways that do not require its ion conducting ability. Instead, MSL10's intracellular N-terminus was shown to have a regulatory function in the induction of programmed cell death, depending on its phosphorylation state. Attempting to uncouple MSL10's ion channel and regulatory functions in plants, we are performing an analysis of several key mutations in the putative pore-lining domain. Several GOF mutants indicate the critical role of hydrophobic residues of TM6 in channel activation. We also have identified LOF mutants with specific changes in particular channel properties, such as single channel conductance and tension sensitivity when tested in *Xenopus* oocytes. We hypothesize that the bulky F553 and F563 residues of MSL10 mediate force transduction from the membrane to the channel's pore. This may occur via interaction with hydrophobic residues of other TMs (like EcMscS F68 and L111) or directly with the fatty acid tails of membrane lipids. Testing these mutants in *in planta* assays will provide insight into the role played by the MSL10 in plant signaling and may identify its interacting partners. In summary, our work is beginning to reveal a dual role of MSL10 in plant physiology and the molecular mechanisms governing its behavior and functions.