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Roles of a putative mechanosensitive plasma membrane Ca^{2+}-permeable channel OsMCA1 in generation of reactive oxygen species and hypo-osmotic signaling in rice

Takamitsu Kurusu,[1,2] Hidetoshi Iida[3] and Kazuyuki Kuchitsu[1,2,*]

[1]Department of Applied Biological Science; Tokyo University of Science; Noda, Chiba, Japan; [2]Research Institute for Science and Technology; Tokyo University of Science; Noda, Chiba, Japan; [3]Department of Biology; Tokyo Gakugei University; Koganei, Tokyo, Japan

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Mechanosensing and its downstream responses are speculated to involve sensory complexes containing Ca^{2+}-permeable mechanosensitive channels. On recognizing hypo-osmotic stress, plant cells initiate activation of a widespread signal transduction network involving second messengers such as Ca^{2+} to trigger inducible defense responses including the induction of transcriptional factors.[1] However, most of the components involved in these signaling networks still remain to be identified. Recently we identified and investigated OsMCA1, the sole homolog of the MCA family putative Ca^{2+}-permeable mechanosensitive channels in rice. Functional characterization of the *OsMCA1*-suppressed cells as well as the overexpressing cells indicated that OsMCA1 is involved in the regulation of plasma membrane Ca^{2+} influx and NADPH oxidase-mediated generation of reactive oxygen species (ROS) induced by hypo-osmotic stress. Here we will discuss possible molecular mechanisms and physiological functions of the MCA protein in hypo-osmotic signaling.

Introduction

Plants respond to mechanical stimuli, such as touch, wind, gravity, pathogen attack and cellular deformation during development.[2] Mechanical stimuli often trigger an increase in cytosolic free Ca^{2+} concentration [$(Ca^{2+})_{cyt}$] mediated by mechanosensitive Ca^{2+} channels located at the plasma membrane and endomembranes.[3-6] However, molecular identity, structure and physiological functions of these mechanosensitive channels are still largely unknown.

Arabidopsis MSL9 and MSL10, homologs of the bacterial mechanosensitive channel MscS, are reported to be required for mechanosensitive channel activity at the plasma membrane of root cells, which are more permeable to Cl^- than Ca^{2+}.[7,8] We recently identified the MCA family proteins including MCA1 (At4g35920), MCA2 (At2g17780), NtMCA1 (AB622811) and NtMCA2 (AB622812) in Arabidopsis and tobacco as putative Ca^{2+}-permeable mechanosensitive channels.[9-11] Ectopic overexpression of *MCAs* enhances Ca^{2+} uptake in roots[9] and cultured cells,[11] as well as $[Ca^{2+}]_{cyt}$ elevation and hypo-osmotic stress-induced gene expression.[9-11] However, components involved in the MCA-mediated signaling pathways have been still mostly unknown.

Recognition of osmotic stress initiates activation of a widespread signal transduction network that induces second messengers and triggers inducible defense responses.[1,12-15] Characteristic early signaling events other than Ca^{2+} influx include protein phosphorylation and ROS generation, most of which are often prevented when Ca^{2+} influx is compromised by either Ca^{2+} chelators or Ca^{2+}-channel blockers, such as La^{3+}.[16,17] These results suggest that regulation of these osmotic signaling events including ROS generation requires Ca^{2+} influx. However, their molecular basis and regulatory mechanisms have remained poorly elucidated.

Intracellular Localization of OsMCA1

The GFP-OsMCA1 protein was localized at the plasma membrane in tobacco BY-2 cells.[18] We also transiently expressed in onion epidermal cells and analyzed the intracellular localization of the GFP-OsMCA1 protein. When GFP alone was expressed, it localized to the nucleus and the cytoplasm (**Fig. 1g–l**). Interestingly, the GFP-OsMCA1 fusion protein was localized specifically targeted to the plasma membrane in patches and at punctuated structures on the cell surface (**Fig. 1a–f**). These fluorescence signals were also observed in tobacco BY-2 cells expressing the NtMCA1-GFP fusion protein.[11] The patchy localization of GFP-OsMCA1 at the plasma membrane appears similar to abscisic acid-binding sites in guard cells[19] and GFP-OsTPC1[20] and may be related to plasma membrane microdomains. The inward rectifying K^+ channel, KST1, also forms clusters in plasma membranes, and its GFP-tagged derivatives

*Correspondence to: Kazuyuki Kuchitsu; E-mail: kuchitsu@rs.noda.tus.ac.jp
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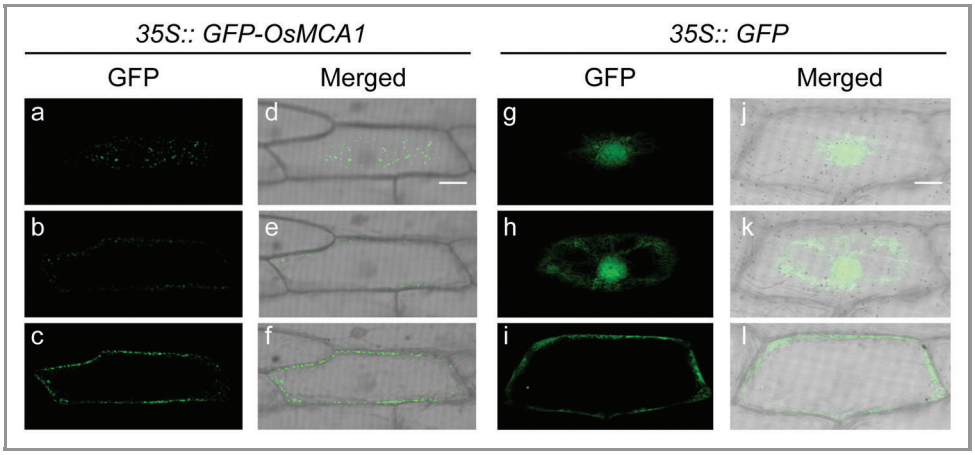

Figure 1. Intracellular localization of OsMCA1. A GFP-OsMCA1 plasmid was introduced into onion epidermal cells by bombardment. (a–f) *35S::GFP-OsMCA1*, (g–l) *35S::GFP*. (a–l) Optical sections of an onion epidermal cell, from the surface (a and d, g and j) to the equatorial plane (c and f, i and l), were obtained by confocal laser scanning microscopy. Fluorescence of GFP (a–c and g–i) and merged with bright field (d–f and j–l). Scale bar: 20 μm.

were observed as patches in both endomembranes and plasma membranes.[21,22] Arabidopsis MCA1 and MCA2 form a homo-oligomer in yeast cells.[23] Plant MCA family proteins may form clusters or complexes with other signaling molecules such as other ion channels in planta.

Roles of OsMCA1 in Ca^{2+}- and ROS-Mediated Hypo-osmotic Signaling

Plasma membrane Ca^{2+} influx induced by various stresses or extracellular stimuli are mediated by at least several types of Ca^{2+}-permeable channels, whose molecular identity are still mostly elusive in plants. Recent studies revealed that temporal patterns of $[Ca^{2+}]_{cyt}$ changes vary among stimuli and molecular nature of Ca^{2+} channels involved.[6,24]

Hypo-osmotic shock as well as treatment with trinitrophenol, an activator of mechanosensitive channels, induce a rapid and transient rise in $[Ca^{2+}]_{cyt}$ predominantly due to plasma membrane Ca^{2+} influx. Both of them are partially impaired in the *OsMCA1*-suppressed cells.[18] In contrast, a major microbe-associated molecular pattern, *N*-acetylchitooligosaccharides, also triggers a $[Ca^{2+}]_{cyt}$ increase with a similar temporal pattern, which is not affected by suppression of *OsMCA1*.[18] These results suggest possible involvement of OsMCA1 as a putative mechanosensitive Ca^{2+}-permeable channel component in the regulation of mechanical stress-triggered plasma membrane Ca^{2+} influx.

Hypo-osmotic shock has also been shown to trigger ROS generation following a $[Ca^{2+}]_{cyt}$ increase in various plant cells.[13,14,17] In rice cultured cells, hypo-osmotic shock-triggered ROS generation is predominantly attributed to NADPH oxidases.[18] Respiratory burst oxidase homologs (Rbohs) possess ROS-producing activity synergistically activated by binding of Ca^{2+} to the EF-hand motifs in the N-terminal cytosolic domain and phosphorylation in rice and Arabidopsis.[25-28] A functional NADPH oxidase AtRbohC/RHD2 affects mechanical stress-induced ROS generation in a Ca^{2+}-dependent manner.[29] Both Arabidopsis MCA1 and ROS generated by AtRbohD and/or AtRbohF have recently been suggested to be involved in the regulation of osmo-sensitive metabolic changes.[30] These findings suggest the following initial plasma membrane responses in response to osmo-stimulation: Activation of the plasma membrane mechanosensitive Ca^{2+}-permeable channels such as MCA family induces the influx of Ca^{2+}, leading to activation of Rboh(s) to generate ROS (**Fig. 2**). The Ca^{2+}-ROS signaling network[27] may play a crucial role in the regulation of downstream events.

In conclusion, OsMCA1 has been shown to be involved in the regulation of plasma membrane Ca^{2+} influx and NADPH oxidase-mediated ROS generation induced by hypo-osmotic stress in

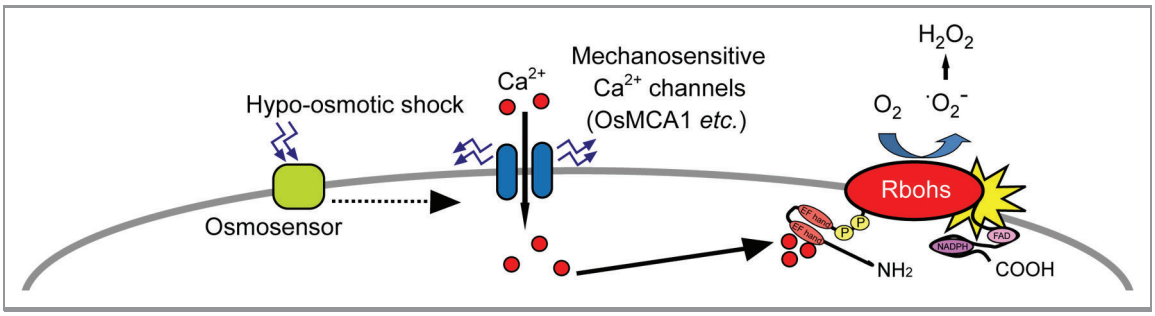

Figure 2. A proposed model for the early plasma membrane responses of rice cells exposed to hypo-osmotic stress. Following the recognition of hypo-osmotic shock by osmosensors or mechanosensitive channels, cells initiate early signaling events including the influx of Ca^{2+}. Respiratory burst oxidase homologs (Rbohs) are synergistically activated by binding of Ca^{2+} to the EF hand motifs and phosphorylation to activate ROS generation.[25-28] Signaling network involving Ca^{2+} and ROS may play a crucial role in induction of stress adaptation. Solid and dotted arrows indicate established and hypothetical links, respectively.

cultured rice cells. These findings shed light on our understanding of mechanical sensing pathways. It should be an important future subject whether OsMCA1 itself also plays a role as a plasma membrane mechanical sensor and/or whether a signal is transduced from unknown mechanical sensor(s) to OsMCA1/Ca^{2+}-permeable channel(s) (**Fig. 2**).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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