COMMENTARY

Shedding light on hypo-osmotic sensing during pollen rehydration

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Organisms open mechanosensitive channels in response to the elevated membrane tension upon hypoosmotic shock. During this process, cytoplasmic solutes are released to relieve cross-membrane osmotic imbalance that will compromise cell integrity. OSCA1 family of mechanically activated ion channels is first identified in plants, and OSCA1.1 (hyperosmolality-induced $[Ca^{2+}]_i$ increase1) regulates Ca^{2+} signals during hyperosmotic stress.[1] TMEM63s are the animal homologs of OSCAs and have pleiotropic physiological functions in mechano- or membrane tension-related processes, including sensation of humidity, thirst, food texture, and regulation of hearing, breathing, and renal function,[2] raising the question if and how OSCAs regulate hypoosmotic stress responses in plants. Now, in a paper published in *Nature*, Pei et al. uncovered that OSCA2.1 and 2.2 function redundantly in converting extracellular water status into Ca^{2+} spiking in pollen during rehydration,[3] thus providing evidence for OSCA-mediated hypoosmotic stress sensing and responses in plants.

REHYDRATION REQUIRES HYPOOSMOTIC STRESS SENSING AND RESPONSES

Water moves across cell membranes from spaces with higher water potential to spaces with lower water potential. All living organisms must monitor water availability in their environment for osmoregulation. The animal cells favor iso-osmotic conditions and suffer hypoosmotic stress, which triggers cell swelling and increases membrane tension. The plant cell wall protects the protoplasts from over-swelling. Therefore, plant cells require hypoosmotic conditions to maintain turgor pressure (about 0.7 MPa in unstressed conditions). Plants accumulate osmolytes under drought, which reduces the intracellular water potential (less than -2 MPa) to counteract the reduction of environmental water potential. Plants also evolved the desiccation-tolerant forms, such as spores, pollen, seeds, or resurrection plants, with extremely low intracellular water potential during their drying stage (less than -100 MPa). Due to the enlarged differences between the intracellular water potential and pure water potential (0 MPa), plant cells may suffer hypoosmotic stress during the rehydration of their desiccation-tolerant forms (Figure 1), or even the rewatering of plants after drought stress. Sensing membrane tension during rehydration or rewatering may protect plant cells from damage caused by over-swelling.

SCREENING FOR THE HYPO-OSMOSENSITIVE OSCAS

OSCA1.1 is identified as a hyperosmolality-gated non-selective cation channel with the following permeability sequence: $K^+ > Ba^{2+} \approx Ca^{2+} > Na^+ = Mg^{2+} = Cs^+$.[1] Interestingly, its closest homolog OSCA1.2 is activated by cell swelling upon hypoosmotic shock in Neuro-2a cells, but not by hyperosmotic shock,[4] suggesting the putative role of OSCA1.2 in hypoosmotic stress responses. Structural evidence indicated that OSCA1.2 forms a new 'proteo-lipidic' pore and functions as homodimers with ion permeation within each subunit.[5] Pei et al. creatively developed a low-salt hypo-osmotic medium for *E. coli* cells expressing OSCAs to study their putative hypo-osmosensing function. They discovered that *E. coli* cells expressing OSCA2.1, OSCA2.5, and OSCA1.3 grow more slowly in a low-salt hypo-osmotic medium, and increases in osmolarity of the medium with sorbitol rescues growth of *E. coli* cells expressing OSCA2.1 and OSCA1.3. Application of Ca^{2+} channel inhibitors La^{3+} and Gd^{3+} also blocks the toxicity of OSCA2.1, OSCA2.5 and OSCA1.3. They further evaluated OSCA2.1 and its closest homolog OSCA2.2 in HEK293 cells and recorded robust Ca^{2+} increases in cells expressing either OSCA2.1 or OSCA2.2. Although the newly developed *E. coli* system identified OSCA2.1, it also raises questions about the distinct performance of OSCA2.2 in *E. coli* and HEK293 cells. These results suggested that OSCA2.1 and OSCA1.3 may be hypo-osmosensitive channels in *E. coli* cells.

OSCA2.1 AND OSCA2.2 ARE VITAL FOR POLLEN GERMINATION AND HYPO-OSMOTIC CA^{2+} SPIKING

OSCA family consists of 15 members in *Arabidopsis thaliana*, while only one TMEM63 exists in *Drosophila* and three members of TMEM63 proteins are present in mammals, which makes it easier to illustrate their physiological functions in animals but not plants. The authors screened individual transfer DNA (T-DNA) mutants of 15 *OSCAs*, but found no significant phenotype in either hypo-osmolarity-induced cytosolic Ca^{2+} concentration (HOSCA) or pollen germination. They generated and evaluated double and triple mutants and found lower pollen germination rates in the *osca2.1/2.2* double mutant without altered pollen viability. They questioned whether OSCA2.1 and 2.2 regulate hypo-osmotic stress-triggered Ca^{2+} signals in pollen, and observed Ca^{2+} oscillation over a 300-min period during seed germination in normal 535 mOsm medium. They observed the first resting phase (RePh1), followed by Ca^{2+} oscillation with small amplitudes ($CaOsc^S$), then a second resting phase (RePh2), followed by Ca^{2+} oscillation with large amplitudes ($CaOsc^L$) which led to tube protrusion, and finally a third resting phase (RePh3) in wild-type pollen. This Ca^{2+} oscillation module is altered in the *osca2.1/2.2* double mutant at 535 mOsm. More importantly, the $CaOsc^L$ gradually propagated towards the germination aperture in wild-type pollen, which is not observed in the *osca2.1/2.2* double mutant. It can be deduced

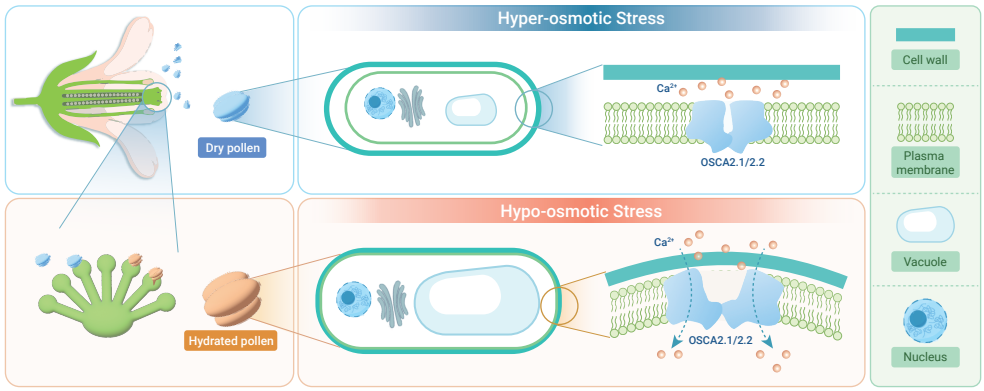

Figure 1. Sensing membrane tension during pollen rehydration by mechanosensitive ion channels OSCA2.1 and 2.2 The mature dehydrated pollen has an extremely low intracellular water potential (less than -100 MPa). After being captured by the stigma, water flows from the stigma to the desiccated pollen grain, which initiates pollen hydration and germination. Plant cells suffer hypoosmotic stress during pollen rehydration, which triggers cell swelling and increases membrane and cell wall tension. The elevated local membrane tension may activate the mechanosensitive Ca^{2+} channels OSCA2.1 and 2.2 and in turn, regulate Ca^{2+} signals during pollen germination.

that the cell wall stiffness is reduced at the germination aperture, leading to elevated local membrane tension, which may activate the mechanosensitive OSCA2.1/2.2.

The elevated local membrane tension results from the cross-membrane osmotic imbalance, which can be repressed by a lower environmental water potential. Indeed, the Ca^{2+} oscillation pattern is substantially altered under the hyper-osmotic condition (680 mOsm),[3] suggesting the failure in the activation of OSCAs. Besides, the Ca^{2+} oscillation defect in the *osca2.1/2.2* double mutant is recovered in a relatively higher environmental water potential (350 mOsm), suggesting the function redundancy of OSCA2.1 and 2.2 with other mechanosensitive Ca^{2+} permeable channels (e.g., other OSCAs) that may vary in the activating tension threshold during pollen germination. Therefore, the authors established that water status in the medium, OSCA2.1 and 2.2, and Ca^{2+} spiking form the sequential hypoosmotic stress responses during pollen germination (Figure 1). However, whether Ca^{2+} spiking is the cause of or is only correlated with pollen germination is still an open question. It must be noted that hypoosmotic stress also triggers many other cellular processes and OSCAs are also permeable to other cations, especially K^+ and Na^+ that are utilized as osmolytes.

WHETHER OSCAS SENSE HYPO-OSMOTIC STIMULI DURING VEGETATIVE GROWTH?

Members of the OSCA family mechanosensitive ion channels may vary in the activating tension threshold, ion selectivity, and conductance. Plants also suffer hypoosmotic stress during rewatering after drought stress or hyperosmotic treatment. The mechanosensitive ion channel MSL10 is required for several known hypo-osmotic shock responses, including a transient cytoplasmic Ca^{2+} elevation, accumulation of ROS, and upregulation of mechano-inducible genes. MSL10 is directly gated by lateral membrane tension and slightly prefers anions, but has not been demonstrated as a Ca^{2+} permeable channel. Whether the large members of OSCAs function together with MSLs in sensing and mediating hypo-osmotic shock responses remains unclear. The high-order *osca1.1/1.2/1.3/1.4/1.5/1.6/1.7* septuple mutant is not defective in the activation of RAF and SnRK2 kinases by hyperosmotic stress. It will be interesting to analyze whether this high-order *osca* mutant is defective in response to hypoosmotic shock at vegetative tissues. Besides the alteration of membrane tension, turgor change is the core signal input for osmotic stress responses in cell wall-containing organisms.[2] Future studies need to resolve whether mechanosensitive ion channels are regulated by protein kinases activated by turgor changes. Thus, this research opens new avenues for studying the sensing of membrane tension and the regulation of Ca^{2+} signaling during pollen germination and characterizes OSCA2.1 and 2.2 as the long-sought hypo-osmosensitive Ca^{2+} channels in plants.

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AUTHOR CONTRIBUTIONS

Y.Z., B.Y., and X.Q. drafted and revised the manuscript. X.Q. prepared the figures.

DECLARATION OF INTERESTS

The authors declare no competing interests.