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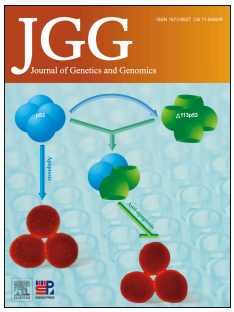

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Sensing of membrane tensions: the pleiotropic functions of OSCA/TMEM63 mechanosensitive ion channels

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Organisms sense and respond to environmental and developmental signals to drive adaptive responses (Kefauver et al., 2020; Yu et al., 2024). These stimuli can be mainly divided into physical and chemical stimuli, and the physical stimuli include light, temperature, sound, vibration, pressure, stretch, shear stress, osmotic stress, and substrate deformation. Some of these physical stimuli trigger physical forces and alter membrane tension, which in turn activates mechanosensitive channels (MSCs) and converts the mechanical cues into biochemical signals. The mechanical activation is thought to be exerted directly via "force from lipid" or indirectly by the "tethered" force from the auxiliary structures such as the interior cytoskeleton and/or extracellular matrix (Kefauver et al., 2020). Activation of these ion channels triggers efflux or influx of ions across membranes, such as K^+, Na^+, Ca^{2+}, and Cl^-, depending on ion gradients across the membranes and the ion selectivity of the ion channels.

MSCs are present in nearly all living organisms. Upon hypoosmotic shock, organisms open MSCs in response to the elevated membrane tension in bacteria, or the volume-regulated anion channels (VRACs) in response to the reduced cytoplasmic ionic strength in animals, a process in which cytoplasmic solutes are released to avoid fast cell expansion that will compromise cell integrity (Buda et al., 2016; Syeda et al., 2016; Helliwell et al., 2021). Therefore, mechanosensitive is not equal to osmosensitive since organisms can sense osmotic stress through multiple signal inputs, including changes in membrane tension and cell fluid volume in all organisms, and changes in turgor in cell wall-containing organisms (Yu et al., 2024). MSCs open in response to a variety of mechanical stimuli,

including local membrane stretching and indentation during cell swelling, squeezing, poking, shearing, and substrate deformation (Kefauver et al., 2020). Among them, the first identified MSCs are the bacterial non-selective MSC of large conductance (MscL) and the MSC of small conductance (MscS), which are activated by the increased membrane tension triggered by the fast volume expansion during hypoosmotic shock (Kefauver et al., 2020). These channels are relatively non-selective and their opening allows the release of cytoplasmic solutes to reduce water influx, preventing fast cell expansion that will compromise cell integrity during hypoosmotic shock (Buda et al., 2016). In Arabidopsis, the MscS-like (MSL) protein MSL8 forms a small-conductance, membrane tension-gated ion channel with a preference for anions; it is required to maintain cellular integrity during pollen hydration and tube growth and may function via directly releasing osmolytes (i.e., mainly ions) in response to the over-elevated membrane tension (Codjoe et al., 2022). MSL10 is directly gated by lateral membrane tension with a slight preference for anions, potentiates hypoosmotic shock and cell swelling-induced responses, and functions at the endoplasmic reticulum–plasma membrane contact sites (Codjoe et al., 2022). MSLs are found in bacteria, archaea, fungi, algae, and plants, but not in animals (Kefauver et al., 2020; Codjoe et al., 2022). Therefore, MscL, MscS, and MSLs are activated by the elevation of membrane tension and their opening passively relieves cross-membrane osmotic imbalances, alters membrane potential, and is required for the adaptation to hypoosmotic shock; since the apoplastic space accumulates high concentration of free Ca^{2+}, opening of these channels may also trigger Ca^{2+} influx into the cytoplasm and in turn mediates multiple downstream responses (Buda et al., 2016; Kefauver et al., 2020; Codjoe et al., 2022).

OSCA/transmembrane 63 (TMEM63) family of mechanically activated ion channels are first identified in plants, and named as reduced hyperosmolality-induced $[Ca^{2+}]_i$ increase 1 (Yuan et al., 2014). OSCAs function in regulating Ca^{2+} signals and stomatal closure in Arabidopsis upon treatments with hyperosmotic stress or the flg22 peptide derived from bacterial flagellin (Yuan et al., 2014; Thor et al., 2020); however, the stomatal responses to low-air-humidity is not defective in the *osca1-2/1.3/2.2/2.3/3.1* quintuple mutant that lacks five *OSCAs* highly expressed in guard cells, or the *osca1.3/1.7* double mutant that is defective in flg22-induced stomatal closure (Hsu et al., 2021). Of particular interest is the flg22-induced BIK1-mediated phosphorylation of OSCA1.3, which enhances the channel activity, a critical requirement for stomatal closure during immune signaling (Thor et al., 2020). OSCA1 is a hyperosmolality-gated non-selective cation channels with preference of cations,

showing the following permeability sequence: $K^+ > Ba^{2+} \approx Ca^{2+} > Na^+ = Mg^{2+} = Cs^+$ (Yuan et al., 2014); the following study reported that OSCA1.1 and OSCA1.2 are MSCs that is comparable to the well-studied PIEZO1 ion channel (Murthy et al., 2018; Zhang et al., 2018). In plants, the plasma membrane is physically linked to the cell wall via cell wall-plasma membrane adhesions. Because hyperosmotic stress peels off the plasma membrane from the cell wall adhesions, it may increase local membrane tension surrounding the cell wall adhesions (Yu et al., 2024). Both hyper- and hypo-osmotic shock alter membrane tension (Yu et al., 2024), and trigger cytosolic Ca^{2+} transient increase in plant cells (Hsu et al., 2021). OSCA1.1 and OSCA1.2 are directly gated by the alteration of membrane tension, either under hyperosmotic stress in plants or upon membrane indentation or stretch in animal cells, but not by changes of ionic strength (Yuan et al., 2014; Murthy et al., 2018; Pei et al., 2022). Moreover, OSCA1.2 from Arabidopsis is an inherently MSC even in liposomes (Murthy et al., 2018), further supporting that it is directly gated by changes in membrane tension.

OSCA family has 15 members in *Arabidopsis thaliana* that can be classified into four clades (Hou et al., 2014; Yuan et al., 2014). Different members of the OSCA family in Arabidopsis have differential responses to biophysically distinct forces triggered by membrane indentation or stretch in PIEZO1-knockout HEK293T cells (Murthy et al., 2018), suggesting these members may vary in the activating tension threshold, ion selectivity, and conductance. Plants must balance cell swelling and cell wall reinforcement via MSCs during environmental challenges such as flooding, rehydration, hypoosmotic shock, and pathogen invasion, or during developmental processes including germination of seeds and pollens, elongation of pollen tubes, and invasive or penetrative growth, in which cells face drastic changes in turgor pressure or cell wall integrity that dramatically alters membrane tension (Codjoe et al., 2022). Although it is challenging, further studies should illustrate expression patterns and subcellular localizations of OSCAs, and generate high-order *OSCA* mutants in plants to characterize their roles in these mechano-related processes. It is also unclear how OSCAs function together with other MSCs in plants, such as MSLs, PIEZO, and Mid1-Complementing Activity 1 (MCA1) and MCA2 (Codjoe et al., 2022). Therefore, plant OSCAs are mechanically activated non-selective ion channels gated by membrane tensions, but their physiological roles require further studies.

OSCA/TMEM63 channels are conserved from yeast to humans, and multiple members of this family from plants, flies, and mammals exert MSC activities (Murthy et al., 2018). OSCAs from Arabidopsis have about 20% identity to mammalian TMEM63s. Cell-membrane indentation elicits

mechanically activated currents in cells expressing OSCA1.1 and 1.2, but not OSCA1.8, 2.3, 3.1, 4.1 and mammalian TMEM63s in the whole-cell patch clamp mode, with a higher threshold (8 μm) compared to mouse PIEZO1 (4–5 μm); however, cell-membrane stretch activates currents in cells expressing OSCA1.8, 2.3, 3.1 and DmTMEM63, MmTMEM63A/B and HsTMEM63A, but not OSCA4.1 and MmTMEM63C, suggesting OSCA4.1 and MmTMEM63C function distinct from other members of the family (Murthy et al., 2018). However, it is also possible that OSCA4.1 and MmTMEM63C are incorrectly folded or not trafficked to the membrane, or they need other components. Besides, the channel conductance of OSCA1.8 and 3.1 is approximately four- and six-fold smaller than OSCA1.1 (Murthy et al., 2018). Moreover, TMEM63A/B and OSCA1.2 are strongly gated by cell swelling triggered by hypoosmotic shock, but not by the cell shrinkage upon hyperosmotic shock, when transiently expressed in Neuro-2a cells (Du et al., 2020), further supporting their functions in sensing the elevation of membrane tension. According to structural evidence, plant OSCAs function as symmetric homodimers with ion permeation within each subunit, and share structural similarity with the mammalian TMEM16 family (Jojoa-Cruz et al., 2018; Liu et al., 2018; ; Zhang et al., 2018; Maity et al., 2019); while animal TMEM63s are monomeric MSCs that are regulated by a lipid-like density located at the cytosolic side of the pore, which needs to be removed to allow ion permeation (Qin et al., 2023; Zhang et al., 2023; Zheng et al., 2023). Generally, OSCA/TMEM63s may directly respond to the local membrane deformation through a lipid-gated mechanism according to structural and liposome evidence (Murthy et al., 2018; Zhang et al., 2023). In contrast to the huge family of OSCAs in plants, only a single TMEM63 member exists in *Drosophila* and there are three members of TMEM63 proteins in mammals, which makes it easier to illustrate their physiological functions in animals.

Indeed, TMEM63 proteins have pleiotropic roles in mechano-related processes, including the sensation of humidity, thirst, food texture, and regulation of hearing, breathing, renal, and brain functions. The mechanosensitive DmTMEM63 senses humidity in *Drosophila* olfactory sensory neurons via converting humidity to mechanical force, and human TMEM63B can substitute for DmTMEM63 to mediate moisture attraction behavior in flies (Li et al., 2022); indeed, the mechanosensitive TMEM63B is required for thirst drive of drinking behavior in mice in the excitatory thirst neurons in subfornical organ (Yang et al., 2024). DmTMEM63 also senses particles in food in *Drosophila* multidendritic neurons through subtle deflections of taste sensilla (Li and Montell, 2021).

Similar to MSCs in bacteria, TMEM63B regulates volume decrease to avoid swelling-dependent cell death in outer hair cells and is required for hearing in mice (Du et al., 2020); TMEM63B is also required for apoptotic remodeling of the postnatal cochlear greater epithelial ridge cells surrounding the inner hair cells, via regulation of thyroxine secretion in thyroid (Ye et al., 2023). Moreover, the mechanosensitive TMEM63A and B are activated by mechanical stretch of alveolar type 2 epithelial (AT2) cells during lung inflation and mediate surfactant secretion from lamellar bodies in mice, and human TMEM63A/B can substitute for MmTMEM63A/B to mediate alveolar expansion (Chen et al., 2023). TMEM63C has not been demonstrated as mechanosensitive; however, it has a role in mediating the glomerular filtration barrier in zebrafish (Schulz et al., 2019). Generally, TMEM63s are also MSCs gated by membrane tension, and their pleiotropic physiological function in animals further supports core functions in monitoring alterations of membrane tension triggered by biophysically distinct forces (Fig. 1).

Mutations in human *TMEM63s* are closely related to neurological and hematological diseases. TMEM63A variants in the pore-forming domain attenuate stretch-activated currents and lead to myelin deficit in infancy with a progressive spontaneous improving course (Yan et al., 2019; Tonduti et al., 2021). Mutations in *TMEM63B* are associated with progressive neurodegenerative brain changes with intellectual disability, and severe early-onset developmental and epileptic encephalopathy, occurring with hematological abnormalities such as macrocytosis and hemolysis that may be associated with defects in hypoosmotic responses; indeed, some TMEM63B variants lead to the inward leak cation currents even in isotonic conditions and impairs Ca^{2+} responses to hypoosmotic stimulation (Vetro et al., 2023). TMEM63C is localized at endoplasmic reticulum, and mutations in *TMEM63C* may be related to focal segmental glomerulosclerosis (FSGS), hereditary spastic paraplegias, and mild intellectual disability (Schulz et al., 2019; Tábara et al., 2022). TMEM63C spans the outer membrane of podocytes of glomeruli that wrap around blood vessels in the kidney and expresses low levels in FSGS patients (Schulz et al., 2019). The physiological function of TMEM63s in hematological health is closely related to their identity as MSCs since red blood cells may be challenged by hypoosmotic shock occasionally. Although these case analyses established the linkage between *TMEM63* mutations and diseases in humans, further investigations with genetically modified animal models are required to clarify the causality and underlying mechanisms. Besides, the correlation between TMEM63s and other MSCs in animals requires further investigation.

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Taken together, OSCA/TMEM63s are bona fide mechanically-activated non-selective ion channels gated by membrane tensions, which convert physical forces from membranes into intracellular electrical and chemical signals, and trigger signaling cascades to mediate physiological processes. Discoveries of OSCA/TMEM63s in plants and animals revealed their critical roles in mechano- or membrane tension-related processes. Because of the low gene redundancy, the physiological function of OSCA/TMEM63s is much clearer in animals than in plants. The future generation of high-order mutants to knock out the entire *OSCA* gene family will help to illustrate their functions in plants. Future studies should clarify how the mechanosensitive OSCA/TMEM63s have pleiotropic roles in plants, insects, mammals, and humans, and identify effective therapeutic strategies targeting TMEM63s.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure legend

Fig. 1. OSCA/TMEM63 channels monitor membrane tension to exert pleiotropic functions.

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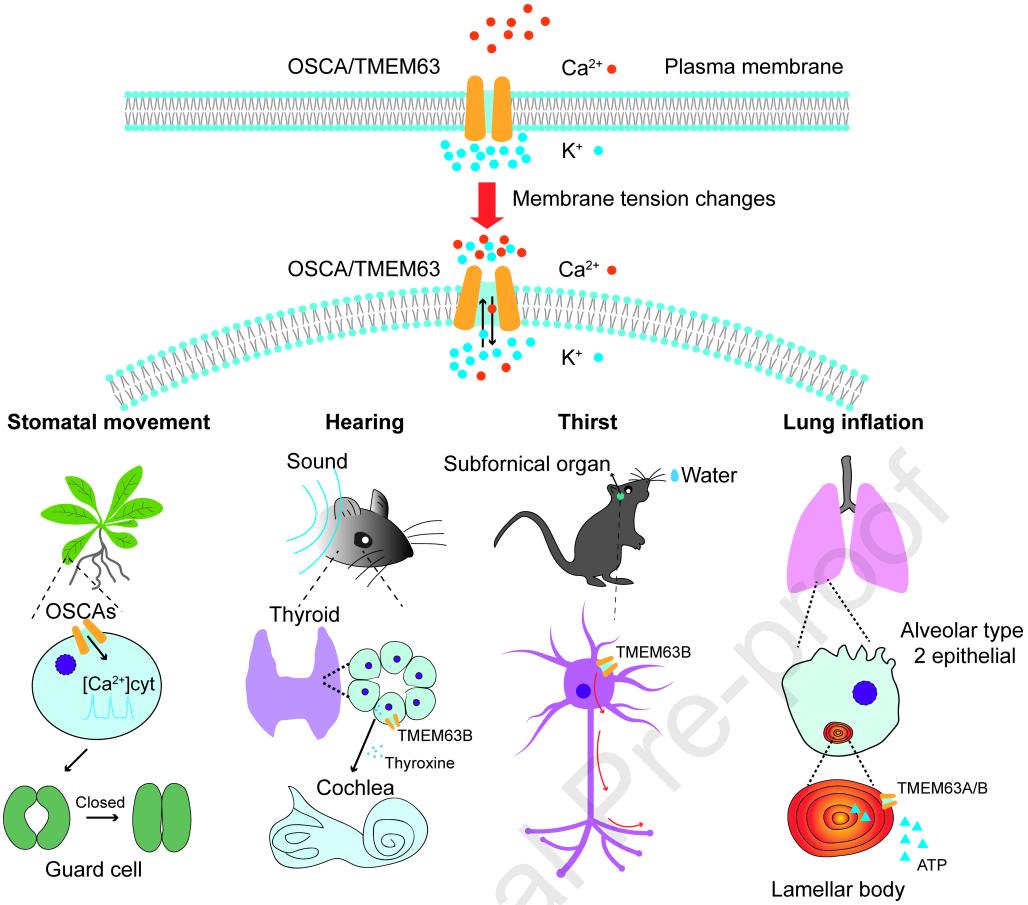

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