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Characterization of Yeast Vacuolar Cation Channel which Confers Salt Tolerance

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Summary

Single copy of transient receptor potential (TRP) channel gene *TRPY1*, are conserved in *Saccharomyces cerevisiae* genome and the gene product localizes in the vacuolar membrane. TRP channel is widely conserved in most eukaryotic cells, except plants, and fungal TRP channel is considered as one of the ancester TRP channels. Although knowledge of TRPY1 has increased, their property and their regulatory mechanism remians to be elucidated. Here we elucidate TRPY1 channel function in vitro and in vivo. Patch clamp recording on TRPY1 in yeast vacuole membrane shows that lumenal Ca^{2+} inhibited TRPY1-mdeiated channel actvity, whereas lumenal Z^{2+} increased the currents. Among eight cysteins facing to cytosolic side, cysteine at position 624 are identified as a target residue for activation of the channel with mercaptoethanol, which is irrespective of the presence of cystosolic Ca^{2+}. TRPY1 was activted by addition of phosphatidylinositol [3] phosphate (PI[3]P) in the cytosolic side but not by those of PI and PI[3,5]P. This was supported by measurment of transient Ca^{2+} increase due to upshock using several yeast mutatns defect for phosphatidylinositol phosphate biogenesis, and by the observation of abnormal vacuole phenotype of the related mutants. The transit cytosolic Ca^{2+} increase was markely eliminated by addition of tubline inhibitors. Taken together, the data represents that tonoplast TRPY1 likely mediate perception of the cytosolic signals elcited by external hyperosmolarity changes and modulate cytocolic calcium signaling through the Ca^{2+} release from vacuole.