No. 1017

Study on Salt Tolerance Conferred by Na Transporters in Bacteria and Plant Cells

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Summary

It is known that HKT/Trk/Ktr and CHX are essential for the adaptation of bacteria and plant cells to salinity stress and high osmolality. In this study, we have examined the cyanobacteria Ktr, Arabidopsis CHX and HKT to gain insight on the mechanism of the salinity stress response. While Synechocystis Na-dependent K uptake KtrABE system, KtrB forms the K-translocating pore, the role of the subunits, KtrA and KtrE for Ktr function remains elusive. Here we characterized the expression of KtrB alone in a K uptake-deficient *E. coli* strain conferred low K uptake activity that was not stimulated by Na. Coexpression of both KtrA and KtrE with KtrB increased the K transport activity in a Na dependent manner. KtrA and KtrE were found to be localized to the plasma membrane in *Synechocystis*. Replacing negatively charged residues facing the extracellular space with residues of the opposite charge increased the apparent Km for K in all cases. However, none of the mutations eliminated the Na dependency of Ktr-mediated K transport. Mutations of residues on the cytoplasmic side had larger effects on K uptake activity than those of residues on the extracellular side. Further analysis revealed that replacement of R262, well conserved among Ktr/Trk/HKT transporters in the third extracellular loop, by Glu abolished transport activity. The atomic-scale homology model indicated that R262 might interact with E247 and D261. Based on these data, interaction of KtrA and KtrE with KtrB increased the K uptake rate and conferred Na dependency.

Flowering plant reproduction requires precise delivery of the sperm cells to the ovule by a pollen tube. We showed that two predicted cation/proton exchangers (CHX) in *Arabidopsis thaliana*, CHX21 and CHX23, are essential for pollen tube guidance. Double mutant pollen grains germinated and grew tubes down the transmitting tract, but the tubes failed toturn toward ovules. Furthermore, chx21 chx23 pollen tubes failed to enter the micropyle of excised ovules. CHX23 mediated K transport, as CHX23 expression in *E. coli* increased K uptake and growth in a pH-dependent manner. We propose that by modifying localized cation balance and pH, these transporters could affect steps in signal reception and/or transduction that are critical to shifting the axis of polarity and directing pollen growth toward the ovule.

We also have constructed the AtHKT1 mutant which has K transport activity, and wheat HKT and salt cress HKT into the Arabidopsis *athkt1* mutant to evaluate the importance of the ion selectivity of AtHKT1. Moreover to monitor the expression pattern of AtHKT1, we introduced the AtHKT1 promoter::GUS gene into the wild type Arabidopsis. Through the above study, we have acquired the knowledge of Na adaptation mechanism and role of the cation-mediated transporters in bacteria and plant cells.