Molecular mechanism of salt- and osmo-tolerance in yeast

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Summary

Adaptation to high salt and high osmolarity conditions is a fundamentally important biological response of all types of cells, ranging from bacteria, fungi, plants, and animals. In yeast, for example, external high salt and high osmolarity conditions activate the HOG (High Osmolarity Glycerol) MAP kinase (MAPK) pathway, which is essential for yeast to adapt to and survive on those conditions.

MAP kinase cascades are conserved signaling modules composed of three sequentially activated kinases (MAPKKK, MAPKK, and MAPK). The HOG pathway consists of two upstream branches, including two independent osmosensors Sho1 and Sln1, and common downstream elements including the Pbs2 MAPKK and the Hog1 MAPK.

The Ssk2/Ssk22 MAPKKKs in the Sln1 branch, when activated, exclusively phosphorylate the Pbs2 MAPKK, but not another yeast MAPKK, Ste7. We found that this was due to an Ssk2/Ssk22-specific docking site in the Pbs2 N-terminal region. The Pbs2 docking site constitutively bound the Ssk2/Ssk22 kinase domain. Docking site mutations drastically reduced the Pbs2-Ssk2/Ssk22 interaction and hampered Hog1 activation by the Sln1 branch. Fusion of the Pbs2 docking site to Ste7 allowed phosphorylation of Ste7 by Ssk2/Ssk22, showing that the docking interaction is an important specificity determinant of MAPKKK-MAPKK interaction.

An analogous MAPK cascade controls cellular response to external hyperosmolarity in mammalian cells. A conserved docking site, termed DVD, was found in the mammalian MAPKKs. DVD site is a stretch of about 20 amino acids immediately on the C-terminal side of the MAPKK catalytic domain. The DVD sites were found to bind their specific upstream MAPKKKs, including MTK1 and ASK1. Mutations in the DVD site strongly inhibited MAPKKs from binding to, and being activated by, their specific MAPKKKs, both *in vitro* and *in vivo*. DVD site mutants could not be activated by various external stimuli *in vivo*. Synthetic DVD oligopeptides inhibited specific MAPKK activation, both *in vitro* and *in vivo*, demonstrating the critical importance of the DVD docking in MAPK signaling.

These studies demonstrate that docking interaction between MAPKKK and MAPKK contributes to both efficiency and specificity of stress-responsive MAPK signaling, in both mammalian and yeast, and perhaps in any eukaryotic organism.