DOCTORAL THESIS

Alternative activation of HOG pathway under hyperosmotic stress and analysis of salt-tolreance in saccharomyces cerevisiae

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Alternative Activation of HOG Pathway under Hyperosmotic Stress and Analysis of Salt-tolerance in *Saccharomyces cerevisiae*

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Abstract

Yeast has evolved with the HOG (high osmolarity glycerol) pathway to survive and adapt to osmotic stress. HOG pathway also belongs to the well-known MAPK signaling pathways. Many components in the HOG pathway have been well studied but the key component, Pbs2p, an MAPKK, has not been shown with additional input. Here, we report that Ssk2p, one of the MAPKKK, can be activated and conduct the HOG pathway independent of Ssk1p under osmotic stress by unidentified mechanisms. We suppose there is another regulator (Temporarily named as X factor) that can bind to the Ssk2p and activate the Ssk2p. We also identified that the binding domain for the unknown X factor is from aa 177 to aa 240 on Ssk2p. The findings can explain early observations that *STL1* and *GRE2* are induced 8- to 38-fold in *ste11*Δ*ssk22*Δ cells but exhibit little induction (<1.7- fold) in *hog1*Δ or *pbs2*Δ strains. Though the alternative activation of Ssk2p under hyperosmotic stress plays important role in non-ionic hyperosmotic stress response, the Sln1p-Ssk1p-Ssk2 cascade serves more efficiently under both non-ionic and ionic hyperosmotic stress than X-Ssk2p cascade. We also observed that the cells of the mutant *ssk2*Δ *(1~240)* lost their sensitivity to the mild osmotic stress (0.2M sorbitol). Furthermore the response is significantly attenuated under osmotic stress compared with that of the wild type Ssk2p. The results indicate that, besides as the activator, the X factor can enhances the activation of Ssk2p by Ssk1p. Or the X factor also plays a scaffolding role for the activation of Ssk2p.

Budding yeast keeps three MAPKKKs, Ste11p, Ssk2p and Ssk22p, to activate one MAPKK Pbs2p to conduct the HOG pathway upon hyperosmotic stress. The three MAPKKKs in the HOG pathway have different roles in salt tolerance. To cope with toxic concentrations of cations, yeast cells need not only the activation of HOG pathway, but also other biological processes. Our results indicate that the

MAPKKKs participate in this process. Ste11p and Ssk2p cope with salt stress caused by sodium equally well, but Ssk22p displays a much poorer capacity. As shown above, X factor can activate Ssk2p independent of Ssk1p and enhance the activation of Ssk2p under osmotic stress. Lacking the binding site (amino acid 177~240aa) for X factor of Ssk2p would reduce the salt-resistance of the *ste11*Δ*ssk22*Δ cells which indicate that salt-resistance requires high level activation of Ssk2p and both Ssk1p and the X factor should be present at the same time and cooperate together.

Our results and others also show that the three MAPKKKs activate the Pbs2p and then the HOG pathway under osmotic stress. To some extent, they are functionally redundant. However, they have distinct activation patterns due to their upstream regulators. The Ste11 branch may not be as sensitive as Sln1-Ssk1-Ssk2 cascade under mild osmotic shock, but keeps osmo-resistance as well as the wild type strain. The Sln1-Ssk1-Ssk2 cascade exhibits both sensitivity and tolerance to the various levels of osmotic stress. The X-Ssk2 branch only responds to the severe osmotic stress, only if concentration is higher than 0.5M sorbitol, KCL or NaCL, and the duration of activation is much shorter. However, the Sln1-Ssk1-Ssk22 cascade displays less sensitivity, slower activation, and lower level of activation capacity even Ssk22p is highly homologous to Ssk2p. Deleting the binding site (amino acid 177~240aa) for X factor of Ssk2p would reduce the sensitivity and response level under hyperosmotic stress. When the different activation patterns are combined together, an activation pattern close to that of the wild type cells can be reached. The differences between the activation patterns of the MAPKKKs depend on their different mechanisms and the protein properties themselves. We conclude that although the MAPKKKs work with some kind of redundancy, it is reasonable for the yeast cells to keep three MAPKKKs to survive under hyperosmotic stress.

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