論文内容の要旨

論文題目：A novel osmosensing mechanism in the yeast HOG MAPK pathway

(出芽酵母 HOG 経路における 高浸透圧感知メカニズム)

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Confronted with extracellular signaling molecules or environmental stresses, organisms need to recognize the signaling molecules/stresses and to respond appropriately to them. When organisms respond to extracellular signal, cellular signal transduction networks have important roles to change the cellular behavior.

In the budding yeast *Saccharomyces cerevisiae*, the High Osmolarity Glycerol (HOG) pathway is essential for adaptation to high osmolarity stress. Activated Hog1 MAPK regulates various aspects of osmo-adaptation, such as the cell cycle, protein translation, gene expression, and the synthesis and intracellular retention of the compatible osmolyte glycerol.

The HOG pathway is activated by either of two functionally redundant upstream mechanisms, termed the SLN1 and SHO1 branches (Figure 1). It is known that an osmosensor for the SLN1 branch is Sln1, a transmembrane histidine kinase that detects turgor changes and generates an intracellular signal. In contrast, neither the osmosensor nor the signal generator for the SHO1 branch was known when I started my investigation, although it was known that a transmembrane protein Sho1 functioned in the upstream of the SHO1 branch.

Recently, it was reported that the transmembrane mucin protein Msb2, whose extracellular domain was highly O-glycosylated, transmitted a signal into a cell following osmotic stress. However, deletion of the *MSB2* gene alone did not inhibit activation in the SHO1 branch. Based on these data, I considered the possibility that another factor that is functionally redundant with Msb2 might be involved in the SHO1 branch.

In this study, I carried out a mutant screen in order to identify and clone the putative factor that is functionally redundant with Msb2. Thus, I found that another mucin-like single pass transmembrane protein Hkr1 acts as a functionally redundant osmosensor in the SHO1 branch. Deletion of the *HKR1* gene or the *MSB2* gene alone conferred no osmosensitivity to yeast cells, whereas activation of the SHO1 branch was completely abolished in a yeast strain in which both the *HKR1* and *MSB2* genes were deleted.

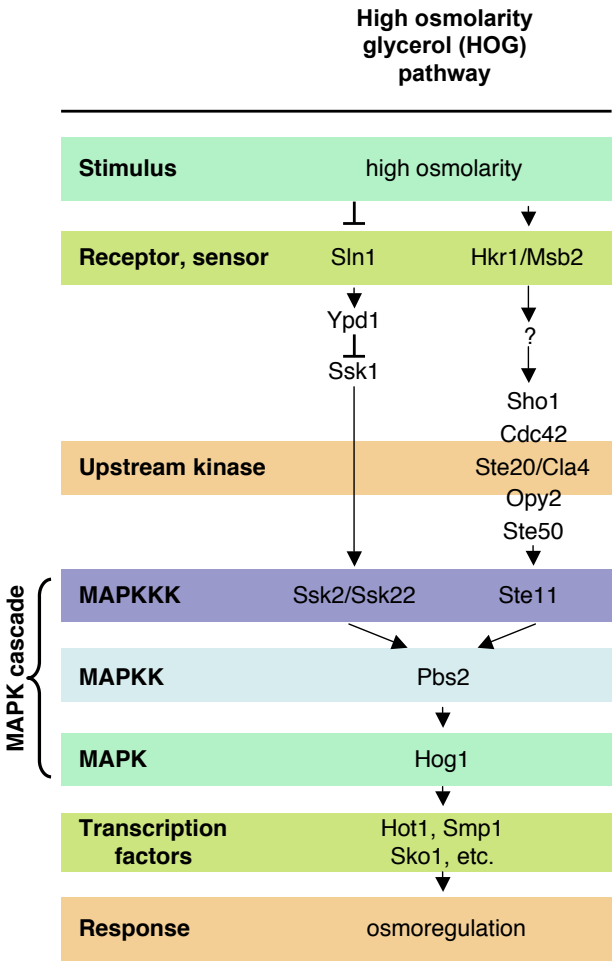

Figure 1 The schematic model of the yeast HOG pathway including recent findings.

Hkr1 and Msb2 are single pass transmembrane proteins of 1802 and 1306 amino acids, respectively. Their extracellular domain has three remarkable similarities. First, both have an extended domain of highly Ser/Thr-rich, which has the potential to be highly O-glycosylated. Second, within the Ser/Thr-rich domain, both proteins have tandem Ser/Thr/Pro-rich repeats that is similar to the mammalian mucin repeats. Third, following Ser/Thr-rich domain, there is a highly homologous non Ser/Thr-rich region between Hkr1 and Msb2, which we termed the Hkr1-Msb2 Homology (HMH) domain. There is no significant sequence similarity between the cytoplasmic domains of Hkr1 and Msb2.

To analyze the contribution of each domain of Hkr1 and Msb2 in osmostress signaling, I constructed various deletion constructs of the *HKR1* and *MSB2* genes and investigated hyperosmotic activation of the SHO1 branch mediated by these mutants. The analysis revealed that the highly-glycosylated extracellular Ser/Thr-rich domain negatively regulates the Hkr1/Msb2 function, whereas the HMH region is essential for signal generation. In contrast, for both proteins, their C-terminal cytoplasmic domain had no obvious signaling function.

Epistasis tests using constitutively-active Hkr1, Msb2, and Sho1 mutants revealed that Hkr1 and Msb2 are the most upstream elements in the SHO1 branch, whereas Sho1 functions downstream of Hkr1 and Msb2. Furthermore, Hkr1 mutants in which extracellular domain are partially deleted exhibited altered kinetic responses to osmotic stimulation, indicating that Hkr1 (and also Msb2) might be an osmosensor that modulates osmosensitivity of yeast. Sho1, although not an osmosensor, is essential to initiate cytoplasmic signaling by transmitting the signal from Hkr1/Msb2.

Unexpectedly, Msb2 can activate the HOG pathway in two different modes. In Mode 1, activated Hkr1 or Msb2 interacts and stimulates Sho1 to generate a cytoplasmic signal. In Mode 2, Msb2 itself generates the cytoplasmic signal. Additionally, Msb2, but not Hkr1, is also involved in cross-talk signaling, which is an inappropriate activation of mating/filamentous growth MAPK pathway following osmotic stress in *hog1*Δ or *pbs2*Δ strain.

To further investigate how Hkr1 and Msb2 activate the downstream pathway, I performed a mutant screen to identify novel regulators in the upstream of the SHO1 branch. For this screen, lethality by constitutively-active Hkr1 signaling in the *ptp2*Δ*ptp3*Δ strain, in which negative regulation of Hog1 MAPK was disrupted, was useful. Suppression of this growth inhibition was an excellent system to sensitively detect deterioration in signaling of the SHO1 branch.

In conclusion, I proved that Hkr1 and Msb2 are the most upstream components of the SHO1 branch and are the likely osmosensors. The signaling model of Hkr1 and Msb2 provides a basis for a novel osmosensing mechanism by membrane-associated mucins. Furthermore, using a newly identified gene *HKR1*, I constructed a good system to detect signaling in the SHO1 branch. This system will be useful for further investigation of the SHO1 branch.