Laboratory of Molecular Genetics (Frontier Research Unit)
遺伝子解析施設（フロンティア研究領域）

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The Laboratory of Molecular Genetics was established for developing various molecular genetic techniques, spreading them to IMSUT investigators and supporting security management related to experiments carried out using recombinant DNA technologies. Since 2017, this laboratory has integrated the Frontier Research Unit for supporting selected investigators to challenge new fields of bio-medical sciences.

Frontier Research Unit

Protein phosphorylation and dephosphorylation are among the most important intracellular signaling mechanisms, and are mediated, respectively, by protein kinases and protein phosphatases. We study various aspects of cellular signal transduction with a particular emphasis on the role and regulation of protein phosphorylation and dephosphorylation in cellular stress responses, using yeast cells.

1. Molecular mechanisms of the osmotic-enhancement of Pbs2 MAP2K phosphorylation by Ste11 MAP3K in the yeast osmo-regulatory HOG pathway

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The yeast MAPK Hog1 is activated by hyperosmotic stress through the High Osmolarity Glycerol (HOG) pathway, and orchestrates an array of osmo-adaptive changes in transcription, translation, cell cycle, and metabolism. The current widely held model of the HOG pathway is as follows. The upstream portion of the HOG pathway is comprised of the functionally redundant SHO1 and SLN1 branches. In the SHO1 branch, osmosensing complexes composed of Sho1, Opy2, Hkr1, and Msb2 activate the MAP3K Ste11. In the SLN1 branch, the Sln1-Ypd1-Ssk1 phospho-relay mechanism is involved in activation of the functionally redundant MAP3Ks Ssk2 and Ssk22 (Ssk2/22). Ste11 and Ssk2/22 phosphorylate the MAP2K Pbs2 at Ser-514 and/or Thr-518. Phosphorylated Pbs2 then activates Hog1. We found that osmostress not only activates membrane-associated osmosensors but also enhances Hog1 phosphorylation by mono-phosphorylated Pbs2. The lack of the osmotic enhancement of the Pbs2-Hog1 reaction suppresses Hog1 activation by basal MAP3K activities and prevents pheromone-to-Hog1 crosstalk in the absence of osmostress, which ensures the appropriate Hog1 activation only under high osmolarity. Recently, we found that osmostress enhances Pbs2 MAP2K phosphorylation by Ste11 MAP3K as well as Hog1 phosphorylation. Ste11 is phosphorylated and activated by the Ste20/Cla4 kinases upon high osmolarity. The constitutively-active Ste11-DDD mutant carries the substitution mutations of all activating phosphorylation sites for Ste20/Cla4 to phosphomimic Asp (S281D, S285D, and T286D), which circumvent the need for Ste20/Cla4 for Ste11 activation. The endogenous-level expression of Ste11-DDD did not induce the phosphorylation of Pbs2 under unstimulated condition, but induced it upon high osmolarity even in the *ste20Δ cla4-ts* mutant. These results strongly suggest that Pbs2 phosphorylation by activated Ste11 is osmotically-enhanced as Hog1 is.

This year, we examined the underlying mechanisms of the osmotic-enhancement of Pbs2 phosphorylation by Ste11-DDD. Possible key factors are the adaptor protein Ste50, which binds to Ste11, and the transmembrane osmosensor Sho1, which binds to

Pbs2. We found that the deletion of either *STE50* or *SHO1* abrogated the osmostress-induced phosphorylation of Pbs2 by Ste11-DDD. We previously indicated that osmostress induces the interaction of Ste50 and Sho1. Induced Ste50-Sho1 association may enhance Ste11-Pbs2 interaction indirectly, leading to Pbs2 phosphorylation by activated Ste11 under high osmolarity. To examine this possibility, we investigated the effects of the binding deficient mutants of Sho1, Ste50 and Pbs2. Osmotically-enhanced Pbs2 phosphorylation by Ste11-DDD was abrogated by not only reduced interactions of Sho1-Pbs2 and Ste50-Ste11, but inhibition of induced Sho1-Ste50 association. Taken these results together, we propose that osmostress induces the structural change of Sho1 to bind to Ste50-Ste11 complex, tethering Pbs2 and Ste11 together to allow activated Ste11 to phosphorylate Pbs2.

2. Acetic acid-induced stress granules function as a scaffolding complex for MEK Pbs2 to activate SAPK Hog1

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Stress-activated protein kinases (SAPKs) respond to a wide variety of stressors. In most cases, the pathways through which specific stress signals are transmitted to the SAPK are not known. We show that the yeast SAPK Hog1 is activated by acetic acid through an intracellular mechanism that does not involve stimulation of the High Osmolarity Glycerol (HOG) signaling pathway beyond its basal level. Rather, acetic acid treatment drives the formation of stress granules, which function as a scaffold to bring Hog1 together with Pbs2, its immediately upstream activating kinase, in a stable assembly that leverages the basal activity of Pbs2 to phosphorylate Hog1. Deletion analysis of stress granule components revealed that the assembly is critical for both the acetic acid-induced activation of Hog1 and its association with Pbs2. Activated Hog1 remains associated with stress granules, which may have implications for its targeting.

Publication

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