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

Professor Yoshinori Murakami, M.D., Ph.D.
Associate Professor Kazuo Tatebayashi, Ph.D.

教 授 医学博士 村 上 善 則
准教授 博士(薬学) 舘 林 和 夫

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 young investigators to challenge new fields of biomedical sciences for specified periods.

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. An AND-gate activation mechanism of the MAPK Hog1 that requires direct osmosensing by Hog1 prevents non-osmotic and cross-talk activation of Hog1

Kazuo Tatebayashi, Katsuyoshi Yamamoto, Taichiro Tomida[2], Satomi Adachi-Akahane[2], Akiko Nishimura[1], Tomomi Takayama[1], and Haruo Saito: [1]Department of Biophysics and Biochemistry, The University of Tokyo, [2]Department of Physiology, School of Medicine, Toho University

The family of protein kinases known as mitogen-activated protein kinases (MAPKs) consists of major intracellular signal transducers in eukaryotic cells. Each MAPK is activated via a three-tiered kinase cascade (MAPK cascade) composed of a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK), and a MAPK. Activated MAPKKK activates a cognate MAPKK by phosphorylating two conserved phosphorylation sites in the flexible activation loop within the catalytic domain. In turn, an activated MAPKK activates a cognate MAPK by phosphorylating the conserved threonine and tyrosine in the TXY motif of the latter's activation loop. MAPK cascades are highly conserved from yeast to mammalian species, so much so that the mammalian p38 MAPK can functionally complement the yeast Hog1 MAPK. A MAPK signal transduction pathway commonly comprises, in addition to the core MAPK cascade, an upstream transmembrane receptor or sensor that detects specific extracellular stimuli, and downstream MAPK substrate molecules (effectors) both in the cytoplasm and in the nucleus. Several different MAPK pathways often co-exist within a cell. In yeast, for example, four MAPKs (Slt2/Mpk1, Kss1, Fus3, and Hog1) are expressed in a cell. If inappropriate crosstalk occurred between two MAPK cascades, a stimulus aimed at activation of only one of these cascades could incite irrelevant or even detrimental responses.

Hog1 is activated by hyperosmotic stress through the High Osmolarity Glycerol (HOG) pathway, and orchestrates osmoadaptive responses including intracellular accumulation of glycerol. The upstream portion of the HOG pathway is comprised of the functionally redundant SHO1 and SLN1 branches. In the SHO1 branch, either of the two related, but distinct, osmosensing complexes, Sho1/Opy2/Hkr1 and Sho1/Opy2/Msb2, activate the MAPKKK Ste11.

In the SNL1 branch, the Sln1-Ypd1-Ssk1 phosphorelay activates the functionally redundant MAPKKKs Ssk2 and Ssk22 (Ssk2/22). Both Ste11 and Ssk2/22 activate the MAPKK Pbs2, which then activates Hog1.

Fus3 is activated by the mating pheromones through Ste11 and the MAPKK Ste7. Although the mating pheromones activate Ste11, which is upstream of Hog1, they do not activate Hog1. Commonly, the lack of pheromone-to-Hog1 cross-talk is explained by the pathway insulation model, which posits that a scaffold protein holds several components of one pathway close together, so that signal flows only within that pathway. Indeed, the components of the Fus3 pathway (Gβγ, Ste11, Ste7, and Fus3) are scaffolded by Ste5 ((Elion, 2001), whereas the components of the Hog1 pathway (Sho1, Hkr1, Msb2, Ste11, Pbs2, and Hog1), are scaffolded by Sho1, Pbs2, Bem1, and Ahk1. To effectively prevent crosstalk, however, the scaffold proteins must hold kinases for significantly longer than the half-lives of their activities, which could be several minutes or longer. Because scaffold complexes are typically unstable, additional mechanisms other than scaffolding of signaling complexes are likely to be necessary to effectively prevent crosstalk.

This year, we found a novel mechanism that would ensure that Hog1 is activated only by osmostress, and not by any other stimulus including mating pheromones. Specifically, we show that Hog1 is activated by an AND-gate mechanism. AND-gate produces an active output only when two independent, active inputs are received. In the case of Hog1 activation, input 1 is generated by the upstream membrane-associated osmosensors. An unexpected finding is that osmostress acts directly on Hog1 itself, and provides the required input 2.

2. Functional roles of the transmembrane interaction between the Sho1 osmosensor and the membrane anchor Opy2 in the activation of the SHO1 branch of osmoregulatory HOG MAPK pathway

Tomomi Takayama[1], Haruo Saito and Kazuo Tatebayashi: [1]Department of Biophysics and Biochemistry, The University of Tokyo.

The SHO1 branch, which is one of the upstream branches of osmoregulatory HOG signaling pathway, employs two related but distinct signaling mechanisms, which are called the SHO1(H) and SHO1(M) sub-branches that respectively utilize the related osmosensing complexes Sho1/Opy2/Hkr1 and Sho1/Opy2/Msb2. Sho1 is composed of four transmembrane (TM) domains and a cytoplasmic SH3 domain that binds to the MAPK kinase (MAPKK) Pbs2. Last year, we demonstrated that Sho1 is an osmosensor in the SHO1 branch of the HOG pathway. Sho1 forms planar oligomers of the dimers-of-trimers architecture by dimerizing at the TM1/TM4 interface and trimerizing at the TM2/TM3 interface. High external osmolarity induces not only structural changes in the Sho1 TM domains, but also the binding between Sho1 and the cytoplasmic adaptor protein Ste50. This Sho1-Ste50 interaction leads to Hog1 activation. In addition to its osmosensing function, Sho1 oligomer serves as a scaffold by binding to the transmembrane protein Opy2 at the TM1/TM4 interface, and to another membrane protein Hkr1/Msb2 at the TM2/TM3 interface. Opy2 is a single-path TM protein of 360 amino acids. Its cytoplasmic region contains three (two major and one minor) Ste50 binding sites that are collectively essential for signaling in the SHO1 branch. As Ste50 is constitutively bound to Ste11, Opy2 recruits the Ste50/Ste11 complex to the plasma membrane. Membrane localization of Ste50 is an important function of Opy2, as an artificial membrane localization of Ste50, by for example using the C-terminal prenylation site of Ras2 (Cpr), suppresses the Hog1 activation defect of *opy2*Δ mutation.

In response to hyperosmolarity, the SHO1 branch activates Hog1 through the Ste20-Ste11-Pbs2-Hog1 kinase cascade. The PAK-like kinase Ste20 is recruited to the membrane by the small G protein Cdc42 as well as by Hkr1 (probably through a hypothesized adaptor protein) or Msb2 through a Bem1 adaptor protein. Similarly, the MAPKK kinase (MAPKKK) Ste11 is recruited to the membrane by the Opy2-Ste50 complex. Ste50 is a cytoplasmic adaptor protein that binds both to Ste11 and to the single-path membrane anchor protein Opy2. Finally, Pbs2 is also recruited to the membrane by Sho1. Thus, both the Ste20→Ste11 reaction and the Ste11→Pbs2 reaction take place on the membrane. One or both of these activation reactions are likely regulated by osmostress; however, no such mechanisms were known.

Last year, we isolated the constitutively-active Sho1 mutant, named Sho1-A/D, which increased the affinity between the TM domains of Sho1 and Opy2. This year, using the mutant, we studied functional roles of the transmembrane interaction between Sho1 and Opy2. First, we revealed that the association of Sho1 and Opy2 was induced at the TM-cytoplasmic boundary by the *SHO1-A30D* mutation using a site-directed cysteine (Cys) chemical crosslinking strategy. Next, we found that enhanced interaction between Sho1 and Opy2 specifically promoted the Ste11→Pbs2 reaction, but not the Pbs2→Hog1 reaction. These results indicated that the transmembrane interaction of Sho1 and Opy2 plays an important role for the activation of the HOG pathway, especially at the step of Pbs2 phosphorylation by Ste11, by holding two signaling complexes: Sho1-Pbs2 and Opy2-Ste50-Ste11, together.