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. Osmostress promotes Ste11-dependent Pbs2 phosphorylation via the osmosensors Sho1 and Hkr1 in the yeast HOG pathway

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To adapt to extracellular hyperosmotic stress, the budding yeast Saccharomyces cerevisiae activates the Hog1 mitogen-activated protein kinase (MAPK), which is activated via the High Osmolarity Glycerol (HOG) pathway. Activation of Hog1 is essential for cellular survival under high-osmolarity conditions and triggers a wide range of adaptive responses, including glycerol synthesis and retention, global transcriptional reprogramming, translational control, and modulation of cell-cycle progression. The HOG pathway thus serves as a well-established model system for understanding stress-responsive MAPK signaling in eukaryotic cells. The HOG pathway consists of a conserved core MAPK cascade and two upstream signaling branches, SHO1 and SLN1, which converge on the MAPK kinase (MAP2K) Pbs2 to activate Hog1. The SLN1 branch is mechanistically well characterized: the transmembrane histidine kinase Sln1 functions as an osmosensor that detects changes in turgor pressure associated with osmotic stress and transmits signals through a phosphorelay system involving Ypd1 and Ssk1 to activate the redundant MAPK kinase kinases (MAP3Ks) Ssk2 and Ssk22. In contrast, the SHO1 branch is less well understood and relies on multiple transmembrane (TM) proteins, including Sho1, Opy2, Hkr1, and Msb2, which are thought to form osmosensing complexes at the plasma membrane. Although these TM proteins have been implicated as osmosensors that initiate signaling in response to osmotic stress, the mechanisms by which osmotic information is transmitted through the SHO1 branch to the core MAPK cascade remain incompletely defined. In particular, it has been unclear which specific steps in the SHO1 signaling pathway are directly regulated by osmotic stress in conjunction with these TM osmosensors, and how such regulation ensures pathway activation exclusively under stress conditions.

Here, we identify at least three distinct osmotically regulated steps in the SHO1 branch of the HOG pathway. As reported previously, one such step is Hog1 phosphorylation by monophosphorylated Pbs2 MAP2K, which is enhanced by osmotic stress independently of TM osmosensors. We further show that osmotic stress promotes Pbs2 phosphorylation by ac-

tivated MAP3K Ste11, and that this process requires the Sho1 osmosensor. This regulation depends on multiple protein–protein interactions, including osmotic stress-induced Ste50–Sho1 association, as well as the Sho1–Pbs2 and Ste50–Ste11 interactions. Together, these interactions suggest a mechanism in which Sho1–Ste50 binding brings the Pbs2–Sho1 and Ste11–Ste50 complexes into proximity, thereby facilitating Ste11-mediated Pbs2 phosphorylation. In addition, we show that Hkr1, but not Msb2, functions as the primary osmosensor in the SHO1 branch. Hkr1 participates in two distinct processes: Pbs2 phosphorylation by activated Ste11 and the upstream activation of Ste11 by Ste20. The latter represents a third osmotically regulated step and is promoted through the association of Hkr1 with Opy2. Together, these findings demonstrate that osmotic stress impinges on the SHO1 branch at three discrete levels, with multiple osmosensing mechanisms operating through osmosensors to ensure robust and specific activation of the SHO1 branch exclusively in response to osmotic stress.

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

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Stress- and mitogen-activated protein kinases (SAPKs/MAPKs) in eukaryotes are activated through protein kinase cascades downstream of cell-surface sensors or receptors and elicit appropriate cellular responses by phosphorylating specific target proteins. In the budding yeast *Saccharomyces cerevisiae*, two major SAPK pathways operate: the Cell Wall Integrity (CWI) pathway and the High Osmolarity Glycerol (HOG) pathway. The HOG pathway is best known for its role in adaptation to hyperosmotic stress, in which plasma membrane osmosensors activate a kinase cascade culminating in activation of the SAPK Hog1 and the accumulation of glycerol to restore osmotic balance. This adaptive response involves closure of the glycerol channel Fps1, metabolic reprogramming to enhance glycerol synthesis, and transcriptional induction of glycerol biosynthetic genes. In addition to hyperosmotic stress, Hog1 is activated by a wide range of unrelated stressors, including temperature shifts, organic acids, oxidative stress, heavy metals, DNA damage, and nutrient limitation. The mammalian SAPK p38 is a functional ortholog of Hog1 and responds to many of the same stimuli, underscoring the evolutionary conservation of this stress-signaling module. The ability of diverse stresses to activate Hog1 raises fundamental questions regarding how distinct stress signals are transmitted to SAPKs and how SAPK activation is coupled to stress-specific cellular responses, particularly given the limited number of defined cell-surface inputs into the HOG pathway.

Here, we demonstrate that the yeast SAPK Hog1 is activated by acetic acid through an intracellular mechanism that does not involve stimulation of the canonical High Osmolarity Glycerol (HOG) signaling pathway beyond its basal activity. Instead, acetic acid induces the formation of stress granules that serve as a scaffold to assemble Hog1 together with its immediately upstream activating kinase, the MAP kinase kinase Pbs2. This spatial organization enables basal Pbs2 activity to efficiently phosphorylate and activate Hog1. Genetic analysis of stress granule components shows that this assembly is required for both acetic acid-induced Hog1 activation and its stable association with Pbs2. Importantly, activated Hog1 remains associated with stress granules, suggesting that stress granules not only promote SAPK activation but may also influence the specificity or localization of downstream signaling. Collectively, these findings identify stress granules as a previously unrecognized platform for SAPK activation and establish a general principle whereby cells achieve stress-specific signaling outputs by repurposing basal pathway activity through stress granules, rather than activating upstream HOG signaling.

Publication

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