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. Two activating phosphorylation sites of Pbs2 MAP2K in the yeast HOG pathway are differentially dephosphorylated by four PP2C phosphatases Ptc1-Ptc4

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The budding yeast *Saccharomyces cerevisiae* survive greatly fluctuating osmotic conditions in natural environment. To cope with an increased external osmolarity, yeast cells elicit a coordinated adaptive response. These adaptive responses are governed by the Hog1 MAP kinase (MAPK), which is activated via the High Osmolarity Glycerol (HOG) signaling pathway. The HOG pathway consists of a core module of three tiers of protein kinases termed a MAP kinase (MAPK), a MAPK kinase (MAPKK, MAP2K), and a MAPKK kinase (MAPKKK, MAP3K). In addition, the upstream part of the HOG pathway comprises the functionally redundant, but mechanistically distinct, SHO1 and SLN1 branches. When yeast cells are exposed to extracellular high osmolarity, the osmosensors in the SHO1 and SLN1 branches independently detect osmostress to activate cognate MAP3Ks. 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 system activates the functionally redundant MAP3Ks Ssk2 and Ssk22 (Ssk2/22). Activated Ste11 and Ssk2/22 phosphorylate and activate the MAP2K Pbs2. Activated Pbs2, in turn, phosphorylates the MAPK Hog1 at T174 and Y176 in its activation loop for its activation.

Unregulated continuous activation of the HOG pathway is deleterious to cell growth, probably by preventing cell cycle progression. Therefore, a mechanism is needed that appropriately inactivates the HOG pathway. Two groups of the protein phosphatases are involved in the HOG pathway inactivation. The first group contains the members of the protein tyrosine phosphatases (PTP), namely, Ptp2 and Ptp3, which dephosphorylate Hog1 at Y176. The second group contains the members of the serine/threonine protein phosphatase type 2 (PP2C), namely, Ptc1, Ptc2, Ptc3, and Ptc4. Of these, Ptc1, Ptc2, and Ptc3 had been proposed as negative regulators of the HOG pathway, because their overexpression rescued the lethality of the *sln1Δ* cell by inhibiting the constitutive activation of the HOG pathway. Furthermore, overexpression of either Ptc1 or Ptc2 inactivated Hog1 *in vivo*, and purified Ptc1 and Ptc2 dephosphorylated

T174 *in vitro*. In contrast, it was concluded that these phosphatases did not inhibit Pbs2 in vivo, because overexpression of either Ptc1 or Ptc2 did not reduce Hog1 phosphorylation at Y176, which served as an indicator of the Pbs2 activity. Thus, it had been proposed that these type 2C phosphatases inactivate Hog1, but not Pbs2. The phosphatases that dephosphorylate Pbs2 had not been identified yet.

The results of overexpression experiments must be regarded cautiously, because at high level of phosphatase expression, non-physiological substrates might be sufficiently dephosphorylated. For that reason, we consider the gene inactivation experiments more reliable. Furthermore, estimating Pbs2 activity indirectly from the extent of Hog1 phosphorylation might be difficult, as the efficiencies of Pbs2 phosphorylating at Hog1 T174 and Y176 may be significantly different.

This year, we examined the phosphorylation status of Pbs2 at the activating phosphorylation sites Ser-514 and Thr-518 (S514 and T518) in various mutants, both in the unstimulated and osmostressed conditions, using the assay method we developed recently. Thus, we found that Ptc1-Ptc4 collectively regulate Pbs2 negatively, but each Ptc acts differently to the two phosphorylation sites in Pbs2. T518 is predominantly dephosphorylated by Ptc1, whereas the effect of Ptc2-Ptc4 could be seen only when Ptc1 is absent. On the other hand, S514 can be dephosphorylated by any of Ptc1-4 to an appreciable extent. We also show that Pbs2 dephosphorylation by Ptc1 requires the adaptor protein Nbp2 that recruits Ptc1 to Pbs2.

2. A novel multifunctional role for Hsp70 in binding post-translational modifications on client proteins.

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The maintenance of a correctly folded proteome (proteostasis) is critical for cell survival. Cells maintain proteostasis under both basal and stress conditions through the expression of molecular chaperones such as Hsp70 and its associated co-chaperone regulators. Importantly, Hsp70 stabilizes and activates of a wide range of signaling molecules including those involved in processes such as DNA damage response, cell cycle control, autophagy, and nutrient sensing. The Hsp70 client proteins involved in these cellular processes tend to be either highly posttranslationally modified (PTMs) or regulate PTMs on other proteins. In turn, these PTMs tightly regulate a multitude of protein properties including subcellular localization, enzymatic activity, and protein interactions.

This year, we have utilized XL-MS to comprehensively understand the Hsp70 interactome. Using this approach, we have gained fundamental new insights into Hsp70 function, including definitive evidence of Hsp70 self-association as well as multi-point interaction with its client proteins. In addition to identifying a novel set of direct Hsp70 interactors which can be used to probe chaperone function in cells, we have also identified a suite of post-translational modification (PTM)-associated Hsp70 interactions. The majority of these PTMs have not been previously reported and appear to be critical in the regulation of client protein function. These data indicate that one of the mechanisms by which PTMs contribute to protein function is by facilitating interaction with chaperones. Taken together, we propose that XL-MS analysis of chaperone complexes may be used as a unique way to identify biologically-important PTMs on client proteins.

Publication

Nitika, Zheng B, Ruan L, Kline JT, Omkar S, Sikora J, Texeira Torres M, Wang Y, Takakuwa JE, Huguet R, Klemm C, Segarra VA, Winters MJ, Pryciak PM, Thorpe PH, Tatebayashi K, Li R, Fornelli L, Truman AW. Comprehensive characterization of the Hsp70 interactome reveals novel client proteins and interactions mediated by posttranslational modifications. *PLOS Biol*. (10):e3001839 (2022)