MAPK Signaling: Sho Business

Dispatch

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Sho1 is a membrane protein in yeast that activates the Hog MAPK signaling pathway in response to high osmolarity. An accumulating body of work has focused on Sho1 as a model to better understand the mechanisms that dictate signaling specificity.

A common theme in the organization of signal transduction within the cell involves the presence of modular interaction domains in the signaling proteins [1]. Such domains frequently bind short peptide motifs in their targets, and thereby facilitate the formation of interaction networks that impinge on many aspects of cellular function [2]. Interaction domains show a wide range of affinities for their ligands, with dissociation constants ranging from low nanomolar to tens or hundreds of micromolar. Two pressing issues involve the degree to which *in vivo* specificity reflects the simple binding properties of such binary interactions, and the extent to which signaling is controlled by the cooperative effects of multivalent interactions, through the use of tandem interaction domains or multiple distinct binding surfaces on individual domains. For these reasons, it is important to unravel the mechanisms that permit the specific activation of a particular pathway by an upstream input, and to determine how other pathways with similar components can be insulated from stimulation by the same input. Several excellent articles have reviewed the importance of scaffolds as a mechanism to segregate the signaling pathways among the MAP kinase (MAPK) cascades found in yeast [3–5]. Here, we discuss recent studies [6–8] focusing on the budding yeast SH3-domain-containing protein, Sho1, that highlight features of signaling pathways that influence the generation of specificity among protein interaction networks.

Interaction Domain Affinity and Negative Selection Aid in Optimizing Signaling Specificity

At least two distinct, unrelated and non-redundant transmembrane proteins, Sln1 and Sho1, regulate adaptation to high salt conditions by activating the high-osmolarity glycerol (Hog) signaling pathway [4,5,9]. While using separate sets of MAP kinase kinase kinases (MAPKKKs), Ssk2 or Ssk22 on the Sln1 branch, or Ste11 on the Sho1 branch, these upstream osmosensing mechanisms converge onto a common MAPKK, Pbs2 (Figure 1A). The primary downstream role of Pbs2 in each branch is to activate the MAPK Hog1 [6], which in turn initiates a cellular response

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involving the efflux of water from the cell and the increased production of glycerol. Whereas Pbs2 is shared by these two osmosensing branches, the Ste11 MAPKKK on the Sho1 branch is shared by other MAPK pathways including one that regulates the mating response. To prevent 'crosstalk' activation between pathways, scaffold molecules and indeed the kinases themselves coordinate a set of interactions that ensure the fidelity of each signaling cascade [4,5,10,11]. For instance, Pbs2 serves both as a scaffold and a kinase in the Hog pathway whereas Ste5 serves as a scaffold for Ste11, Ste7 and Fus3 kinases in the mating pathway (Figure 1A) [5].

Activation of the Hog pathway by the Sho1-mediated signaling branch requires the localization of Pbs2 to the membrane, which is facilitated by the interaction of an SH3 domain located on Sho1 with a proline-based motif found on Pbs2 (Figure 1A) [12–14]. The binding affinity of this interaction ($K_D = 0.8–1.3$ μM) is modest, though characteristic of the majority of physiologically relevant SH3 domain interactions. Given the 27 other SH3 domains encoded in the yeast genome [2], several questions arise: with only a relatively weak affinity, is the Sho1 SH3 domain–Pbs2 motif interaction specific and, if so, is a precisely tailored interaction required to maintain specificity in signaling at the pathway level?

Studies by Zarrinpar *et al.* [6] and Marles *et al.* [7] have recently examined the role of the Sho1 SH3 domain–Pbs2 interaction in determining the specificity of the osmosensing signaling response. Using yeast expressing panels of either mutant Sho1 SH3 domains [7] or altered Pbs2 motifs [6], these authors demonstrated that the strength with which the SH3 domain binds the Pbs2 motif correlates with the ability to activate the Hog pathway and resist high osmolarity [6,7]. Moreover, as the affinity of the SH3 domain interaction for Pbs2 diminishes, Hog signaling is compromised and the level of inappropriate crosstalk to the mating pathway in response to high osmolarity increases (Figure 1B) [7]. Accordingly, the magnitude of the free energy change of the SH3-mediated interaction exhibits a linear correlation with the ability to maintain signaling specificity. Thus, an appropriate affinity between Sho1 and Pbs2 is required for efficient and exclusive signaling toward the Hog pathway and depends on Pbs2 localization at the membrane. This observation begs the question as to how the affinity of the Sho1 SH3 domain for Pbs2 relates to the mutual selectivity of these two proteins for one another against a background of numerous SH3 domains and proline-rich motifs expressed in the same cell?

Since binding specificity is a relational property that corresponds to the ability of a protein to discriminate between multiple ligands, a meaningful understanding of specificity requires the examination of such potential competing targets. Zarrinpar *et al.* [6] explored this problem by replacing the SH3 domain of Sho1 with 26 other yeast SH3 domains or 12 non-yeast SH3 domains. Importantly, none of the yeast SH3 domains could complement osmoresistance mediated by the

Figure 1. Components of the various yeast MAPK signaling pathways.

(A) High salt conditions activate various MAPK cascades mediated by at least three distinct membrane proteins: Msb2 (not shown), Sln1 and Sho1. In the Sho1 branch, high salt conditions result in localization of Pbs2 to the membrane mediated by a proline-based motif on Pbs2 and an SH3 domain on Sho1. Pbs2 binds the MAPKKK Ste11 which is phosphorylated by the PAK-like kinase Ste20 which is itself recruited to the membrane by activated Cdc42. Activated Ste11 then phosphorylates Pbs2 which in turn activates the downstream Hog pathway. Cross-talk between pathways is, at least in part, limited by various scaffold proteins such as Pbs2 in the Sho1 pathway or Ste5 in the mating pathway. The current studies (discussed in the text) show that the Sho1 SH3 domain binds with surprisingly high specificity to a Pbs2 motif (numbered 1) and that this specific interaction prevents physiologically unfavorable interactions with other yeast SH3 domains [8]. Contributing to the efficiency of this signaling pathway are additional regions of Sho1 including residue insertions in the SH3 domain (numbered 2) [7] and a region in the cytoplasmic tail of Sho1 (numbered 3) that directly binds Ste11 [8]. (B) In the absence of Pbs2 or Hog1, high salt conditions result in cross-talk toward the mating pathway due to the activation of the MAPKK Ste7 by Ste11 in a Sho1-dependent manner (red arrow). Reducing the Sho1 SH3 domain-Pbs2 affinity also results in a concomitant decrease in Hog signaling and an increase in cross-talk responses likely due to the failure to efficiently localize Pbs2 to the membrane [7].

A Common signaling elements segregated on separate scaffolds

B Crosstalk signaling in the absence of Pbs2 or Hog1
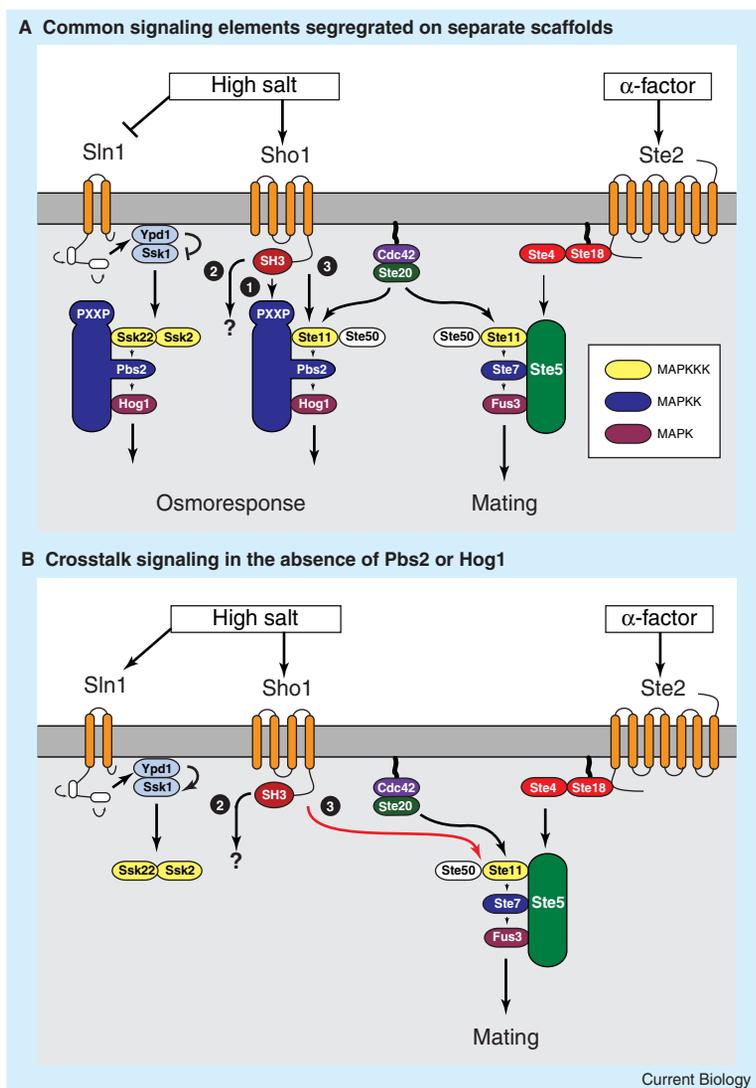
Hog pathway. In contrast, 6 of the 12 non-yeast SH3 domains could rescue osmoresistance, a finding that correlates with their ability to bind the Pbs2 motif *in vitro*. Interestingly, while altering the wild-type Pbs2 proline-rich motif could cause either increased or decreased binding affinity for the Sho1 SH3 domain, all such changes resulted in cross-reactivity toward other yeast SH3 domains [6]. Together, the results suggest that the Sho1 SH3 domain has not been tailored so much for binding affinity toward the Pbs2 motif, but instead the Sho1–Pbs2 interaction has been optimized for binding the Sho1 SH3 domain to the exclusion of other yeast SH3 domains. To explain this phenomenon, the authors suggest that during the course of yeast evolution, recognition of the Pbs2 motif by other yeast SH3 domains has been eliminated through a mechanism of negative selection. Non-yeast SH3 domains would be exempt from such negative selection thus potentially explaining their cross-reactivity to the Pbs2 motif.

In testing the biological importance of maintaining a highly specific interaction in this domain–motif pair, yeast expressing Pbs2 bearing a wild-type or promiscuous SH3 binding motif were found to out-compete cells expressing a mutant Pbs2 unable to bind any yeast SH3 domain under conditions of high salt [6]. In contrast, yeast strains expressing Pbs2 bearing a promiscuous SH3 binding motif were unable to compete with wild-type and non-interacting Pbs2-expressing yeast strains under conditions that did not require the high osmolarity response pathway. These results revealed a fitness defect in the promiscuous yeast strain emphasizing the notion that pleiotropic SH3-domain binding by the Pbs2 motif is functionally disadvantageous and thus disfavored in evolutionary terms.

Building Complexes Using Domains and Docking Sites

Observations by both groups suggest that the Sho1 SH3 domain interaction with the Pbs2 proline motif may not be the only mechanism contributing to the formation of the osmosensing signaling complex. First, yeast expressing a mutant Sho1 SH3 domain unable to bind the Pbs2 motif can still induce a strong crosstalk response; also, replacing the Sho1 SH3 domain with a

Fyn SH3 domain engineered to bind Pbs2 with a similar affinity to that of the Sho1 SH3 domain failed to rescue Hog1 pathway activation to a wild-type level [7]. Second, deleting the Sho1 SH3 domain [7,8] or replacing it with the engineered Fyn SH3 domain severely reduced the mating crosstalk response activated by high osmolarity [7]. Finally, in a separate study [8], the Lim lab posits that Sho1 may have an additional binding site that enables direct binding to the MAPKKK Ste11, since Ste11 is able to activate the crosstalk mating response in a Sho1-dependent manner even in the absence of the scaffolding role of Pbs2. Taken together, a more complicated scenario emerges in which multiple binding interactions may be involved in coordinating complex formation around Sho1.

Upon closer analysis, both labs subsequently identified several additional regions in Sho1 that are responsible for efficient Sho1-mediated pathway stimulation and robust crosstalk activation. Marles *et al.* [7] demonstrated that mutation of two atypical sequence features within the Sho1 SH3 domain (a two residue insertion in the RT-Src loop and a basic residue conserved among other yeast Sho1 homologs) severely reduced crosstalk responses mediated by Sho1, independent of Pbs2 motif recognition. Importantly, the authors also demonstrated that these SH3 domain residues are necessary for activation of a more physiologically relevant Sho1-mediated pathway induced by protein glycosylation defects [7]. The exact mechanism by which these sequences exert these effects has yet to be ascribed but these data suggest that the Sho1 SH3 domain may have additional functional regions that aid in the activation of Sho1-mediated pathways.

Along the same lines, Zarrinpar *et al.* [8] have identified an additional region between the Sho1 transmembrane region and the SH3 domain that is essential for the crosstalk response with the mating pathway but is dispensable for Hog-mediated osmoresistance. Furthermore, this region has a direct binding site for Ste11 and can, independently of Pbs2, mediate the mating crosstalk response. These newly identified docking sites for components of the MAPK pathways may provide an additional layer of signaling specificity by coordinating a series of interactions that contribute cooperatively to the overall binding energy of the signaling complex. As pathway specificity is also defined by the mutual dependency of kinase activation and component recognition, an important dimension that will require attention is the ordering of the formation of this complex which probably relies on the use of multiple cooperative, allosteric and mutually exclusive interactions [8]. Thus, while the interaction of the Sho1 SH3 domain with Pbs2 appears sufficient to maintain signaling specificity toward the Hog pathway, these data suggest Sho1 and Pbs2 have co-scaffolding roles that probably aid in mediating efficient downstream signaling [8].

Lessons for Signaling in Complex Organisms
Studies performed in yeast elegantly highlight some of the biochemical mechanisms that contribute to specificity in signaling. One of the key findings described here is the idea that a relatively low-affinity protein–protein interaction can nonetheless yield specific recognition *in vivo*; in the case of Sho1, this apparently depends on a combination of positive recognition of the physiological binding motif and negative selection for non-physiological partners. Additional mechanisms that contribute to specificity in this system include the use of scaffolds to segregate common signaling components toward discrete pathways and, potentially, the use of cooperative interactions mediated by a combination of modular interaction domains, motifs and docking sites to bolster the co-localization of signaling molecules. Further analysis of multi-protein signaling complexes in various systems, including mammalian cells, raises the possibility that such mechanisms are likely to be a more general feature in determining the correct flow of information through regulatory pathways [15].

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