

Shaping specificity in signaling networks

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Signaling pathways are frequently connected through shared intracellular molecules, yet they manage to maintain remarkable specificity to distinct stimuli. A new study identifies mechanisms to explain how this specificity is shaped for MAP kinase modules within the yeast signaling network.

A characteristic feature of cellular signaling in eukaryotic cells is that components are frequently shared among pathways, providing a potential for cross-talk. However, this can also lead to an inappropriate response, if stimulus-specific signals transmitted through one pathway inadvertently cross-activate the other(s). A report on page 409 of this issue by McClean *et al.*¹ describes a mechanism, referred to as mutual inhibition, that eliminates unwanted interaction between two yeast mitogen-activated protein (MAP) kinase pathways, even when shared molecular signals for both pathways are simultaneously activated.

Spatial and temporal insulation

Several known mechanisms can enable signaling pathways with shared components to respond specifically to any one stimulus. Spatial insulation can be achieved by localizing the pathways to different cellular compartments or by incorporating the shared component into distinct macromolecular complexes through scaffolding molecules. Functionally sophisticated and evolutionarily fine-tuned, highly specific scaffolding has a key role in stimulus-specific signaling through yeast MAP kinase modules^{2–4}. Insulation can also be temporal, if the signals generate noncoincident activation of the shared element (as is the case for the Fus3 homolog Kss1 in yeast⁵) and is often due to feedback loops⁶. In addition, as has been shown for yeast pheromone signaling⁷, cross-inhibition can eliminate the activation of other pathways.

Specificity of MAP kinase modules

In *Saccharomyces cerevisiae*, responses to pheromones or to increased osmolarity of the surrounding environment are both mediated through cell surface molecules that converge on MAP kinase cascades with a shared MAP kinase kinase called Ste11 (Fig. 1a). Activation of the α -pheromone receptor leads to the dis-

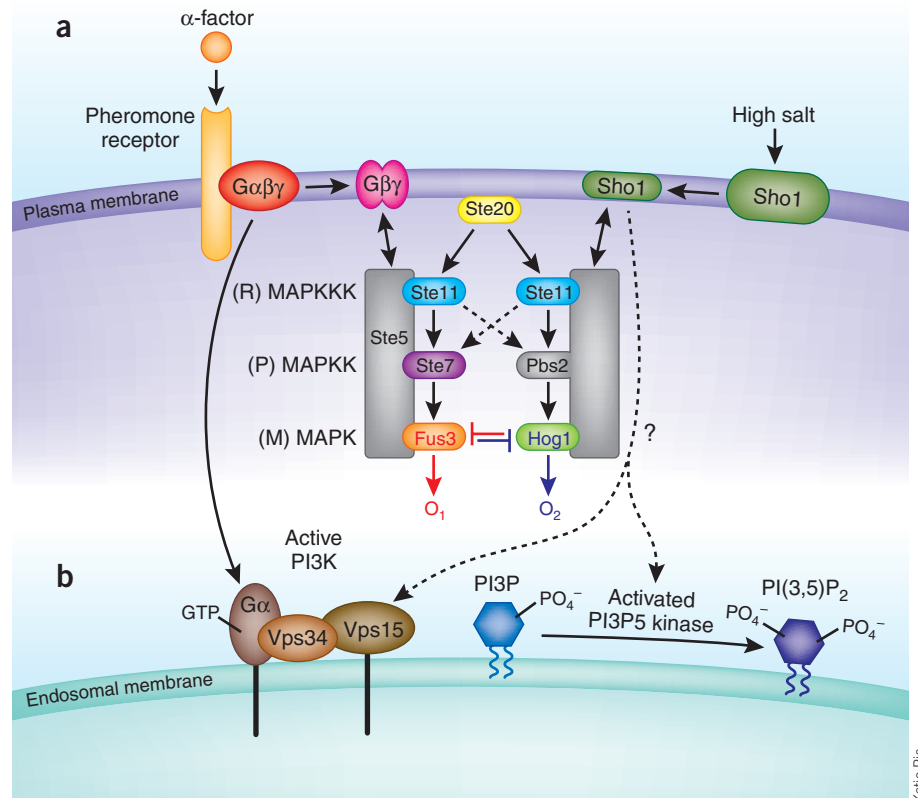


Figure 1 Interaction of MAP kinase pathways in *S. cerevisiae*. (a,b) Pheromones and hyperosmolarity exert an effect on signaling complexes in both the plasma membrane (a) and the endosomal membrane (b). For the MAPK modules tethered to the plasma membrane (a), specificity and bistability of signaling is ensured through scaffolding and mutual inhibition. In the endosomal membrane (b), both pheromone-induced phosphatidylinositol 3 kinase (PI3K) and hyperosmolarity-induced PI3P5 kinase are present, suggesting a possible integration of the two stimuli.

sociation of a coupled heterotrimeric guanine nucleotide-binding protein (G protein) to activated G α (Ste2) and G $\beta\gamma$ (Ste4/18) subunits, the latter tethering the MAP kinase module Ste11/Ste7/Fus3 to the plasma membrane through its interaction with their scaffold protein, Ste5 (Fig. 1a). The osmolarity pathway is activated through the sensor protein Sho1 (and in part through another molecule called Sln1)¹. Under high-salt conditions, Sho1 undergoes a conformational change that leads it to tether the MAP kinase module Ste11/Pbs2/Hog1 to the plasma membrane (Fig. 1a). Initially, both modules are activated by the membrane-bound Ste20

kinase which activates a MAP kinase kinase that in turn activates one or more MAP kinases, which then regulate their respective downstream target molecules (denoted O₁ and O₂)^{1,2} (Fig. 1a). Despite sharing a common Ste11 molecule, the response remains specific: activation of the hyperosmolar pathway does not initiate a pheromone response and vice versa.

Is this MAP kinase specificity based on spatial or temporal isolation of Ste11, or is it based on mutual cross-inhibition downstream of Ste11? The key to answering this question is to study the response when both signals are present simultaneously. For this, McClean *et al.*¹ first considered a generic mathematical model representative of

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the two MAP kinase modules (Fig. 1a). The first step in each pathway is the phosphorylation of the proteins R_1 or R_2 (here, both are Ste11) in response to their respective signals, followed by two consecutive phosphotransfer reactions ($R \rightarrow P$ and $P \rightarrow M$), with the output proportional to the concentration of phosphorylated proteins M_1 or M_2 (here, Fus3 or Hog1). These activation cascades are modulated by two types of cross-reactions: phosphotransfer from R_1 to P_2 and R_2 to P_1 (cross-activation) and dephosphorylation of M_1 by M_2 and vice versa (cross-inhibition).

Maintaining signaling specificity within this circuit seems to require the reduction of cross-activation to zero. Indeed, McClean *et al.*¹ find numerically that for a range of reaction parameters, both outputs are active when only one signal is present. Yet, when cross-activation is weaker than the activation of the correct pathway, and cross-inhibition is stronger than a critical value, one of the two outputs is predominant, even in the presence of both signals. Notably, the simulations show that even with the same reaction parameters and signal strengths, either output can be prevalent, indicating that the system is bistable (Fig. 1). Bistability goes hand in hand with history dependence⁸, meaning that the order of addition of stimuli affects the final response.

McClean *et al.*¹ continue by providing experimental verification of a history-dependent, bistable, switch-like behavior, thus providing key support for the role of mutual inhibition in yeast MAP kinase signaling. Using green fluorescent protein (GFP) and red fluorescent protein (RFP) as transcriptional reporters of pheromone and hyperosmolar-specific activation, respectively, the authors show that each responding cell expresses either GFP or RFP when exposed both to pheromone and to hyperosmolarity-inducing

sorbitol. Also, the fact that fewer cells respond to α -factor in a culture pre-exposed to sorbitol than in a culture pre-exposed to the pheromone demonstrates that the pathways show history-dependent bistable behavior. Finally, the loss of response specificity upon exposure to sorbitol in the cells carrying the Fus3^{D63S} mutant⁷ confirms that this mutant form of Fus3 resists inhibition by the osmolarity cascade⁷ and supports the notion that yeast MAP kinase signaling specificity is maintained both by scaffolding and mutual inhibition.

Integration of multiple signals

An apparent evolutionary advantage of the above molecular circuit is that it enables exploratory behavior in the face of conflicting signals: the pursuit of different courses of action by identical yeast cells allows for a better survival of the population, and the use of shared components provide the lowest biosynthetic cost for them. In addition, a dynamic regulation of cross-talk would allow a more flexible modulation of signaling specificity than that provided by scaffolding alone. This can prove useful for cells incorporating information from specific external stimuli and from continuous monitoring of their own internal state (for example, morphology, internal structure and stress level). Together, both external and internal states may influence signaling through these circuits and the subsequent cellular response.

Are yeast cells completely specific in their overall response to a specific stimulus, or do they modulate it when more than one signal is present? Evidence for the latter is observed for the above pathways. After pheromone receptor activation (Fig. 1a), G α also translocates to the endosomal membrane (Fig. 1b), where it interacts with the catalytic (Vps34) and regulatory

(Vps15) subunits of the sole yeast phosphatidylinositol 3-kinase (PI3K) and activates PI3K-catalyzed production of phosphatidylinositol 3-phosphate (PI3P)⁹. This G protein signal seems to be fundamentally different from that of its plasma membrane-bound form⁹. PI3P can be further converted to phosphatidylinositol 3,5-bisphosphate (PI3,5P₂) by an endosomal PI3P5-kinase whose activity is rapidly upregulated by hyperosmotic stimuli¹⁰ (Fig. 1b), suggesting an apparent mechanism for integrating the two signals. Thus, while plasma membrane MAP kinase modules clearly select between initial pheromone or hyperosmolarity signaling (Fig. 1a), a counterpart endosomal mechanism (Fig. 1b) may partly integrate them, leading to a modulated overall response. In general, spatially separated signaling complexes, whose distinct and time-dependent functions are shaped by the cell's internal state, may produce an ever-shifting dynamic balance between response specificity and promiscuity in eukaryotic cells.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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Think globally, act locally

Valerie Reinke

In the nematode *Caenorhabditis elegans*, dosage compensation is mediated by a subtle twofold downregulation of both X chromosomes. A new study provides a significant advance in our understanding of how the X is targeted for dosage compensation and how this global regulation is integrated with regulation of the expression of each gene.

Dosage compensation is the process by which sex chromosome gene expression is equalized between sexes. Although the mechanisms

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underlying dosage compensation are diverse among species, common obstacles must be surpassed in order for dosage compensation to be effective. These obstacles include determining the correct sex in which to establish dosage compensation, recognizing and targeting the appropriate sex chromosome (usually the X) and instituting a relatively uniform global regu-

lation over an entire chromosome. On page 403 of this issue¹, Sevinc Ercan and colleagues provide insight into how two of these hurdles are overcome during dosage compensation in *C. elegans*.

The *C. elegans* XX hermaphrodite genome contains a double dose of X-linked genes relative to XO males. This difference is