

Control of MAP kinase signaling specificity or how not to go HOG wild

George F. Sprague, Jr.

Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403-1229 USA

Mitogen-activated protein kinase (MAPK cascades; also called ERK cascade, for extracellular signal-regulated protein kinase) are at the center of many signal transduction pathways in eukaryotic cells. These cascades are composed of a trio of sequentially acting protein kinases that relay an extracellular physiological signal to the targets that orchestrate the appropriate cellular response. A given mammalian cell type typically contains multiple MAPK cascades (for review, see Robinson and Cobb 1997), which raises a number of intriguing questions. Is each cascade dedicated to a specific physiological response? What ensures the specificity of action of each cascade? Do distinct MAPK cascades share some components? What mechanisms restrict or regulate cross talk between distinct pathways? These issues can be difficult to decipher in mammalian cells, but recent studies in yeast have shed light on these questions. Strikingly, these studies have revealed that the MAP kinases can function as specificity factors.

Yeast MAPK pathways

Yeast cells contain five MAPK cascades that orchestrate responses to different physiological stimuli. One of these cascades operates in cells undergoing meiosis and regulates spore formation. The other four cascades operate in vegetative, mitotically active cells. Of these cascades, two control developmental events—mating and filamentation—and two control response to solute concentration—the HOG pathway orchestrating response to high osmolarity and the MPK pathway orchestrating response to low osmolarity (for recent reviews, see Herskowitz 1995; Leberer et al. 1997; Levin and Errede 1995; Madhani and Fink 1998). This article will concentrate on recent reports that reveal a greater sharing of components among the mating, filamentation, and HOG pathways than previously appreciated (Cook et al. 1997; Madhani et al. 1997; O'Rourke and Herskowitz 1998). More important, these reports also point to two MAPK proteins that limit cross talk among these pathways and thereby ensure specificity. Moreover, these two MAPKs rely on different mechanisms to fulfill their roles as specificity factors.

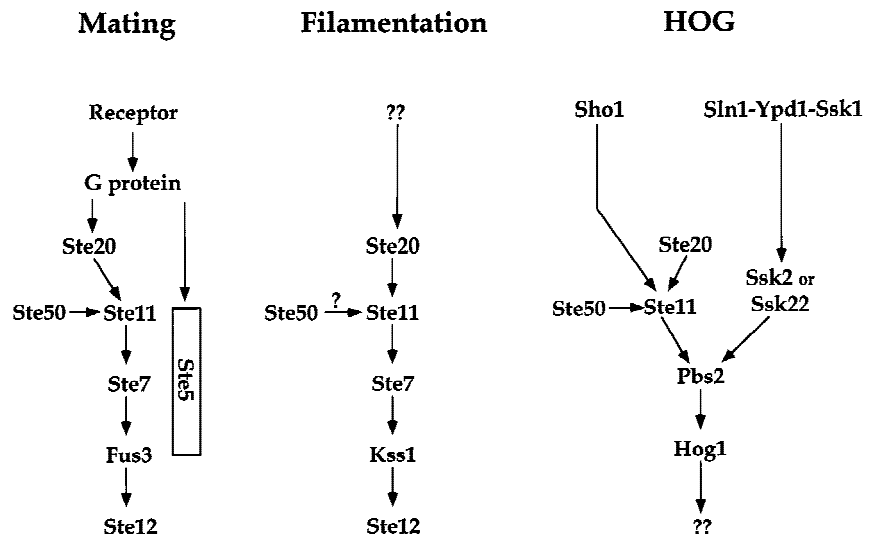
The mating pathway is activated in haploid α or a cells by binding of peptide pheromone to cell-surface receptors, an event that in turn activates a trimeric G protein (Fig. 1). In ways that are incompletely understood, the G protein then activates the mating pathway MAPK cascade consisting of Ste11p, a MAPK kinase kinase (MAPKKK), Ste7p, a MAPK kinase (MAPKK), and Fus3p, a MAPK. The activated G protein binds to both Ste20p, a PAK-related protein kinase, and Ste5p, a scaffold protein that binds each component of the MAPK cascade. Because both Ste20p and Ste5p interact with Ste11p, it is likely that the G-protein interactions with Ste20p and Ste5p each contribute to activation of the MAPK cascade. Fus3p then phosphorylates target proteins that direct the mating program. For example, the Fus3p target Ste12p is a transcription factor that regulates transcription of mating pathway inducible genes.

The filamentation pathway shares a number of components with the mating pathway. The signal(s) that activates the filamentation pathway has not been identified with certainty. In diploid cells, which exhibit pseudohyphal formation in response to activation of the filamentation pathway, low nitrogen may be an activating condition. In haploids, activation of the filamentation pathway leads to invasion of the underlying agar, but the inducing signal is not known. Although the initial components of the filamentation pathway have not been identified, signal transmission requires some mating pathway components: Ste20p, Ste11p, Ste7p, and Ste12p, the latter required for transcription induction of the filamentation pathway gene set. Filamentation does not require other mating pathway components, specifically the receptors, the trimeric G protein, the scaffold protein Ste5p, and the MAPK Fus3p. Until recently, the MAPK that functions in the filamentation pathway was not known. However, a nice piece of genetic detective work revealed that Kss1p, a MAPK previously thought to be redundant with Fus3p in the mating pathway, is in fact dedicated to the filamentation pathway (see below; Cook et al. 1997; Madhani et al. 1997). The same detective work showed that Fus3p is dedicated to the mating pathway and is a specificity factor that ensures that the mating rather than filamentation program is activated by pheromone.

The HOG pathway also shares a component with the

E-MAIL gsprague@molbiol.uoregon.edu; FAX (541) 346-4854.

Figure 1. Three MAPK signaling pathways in the yeast *Saccharomyces cerevisiae*. The components of three MAPK signaling pathways are shown (see text for details). In the HOG pathway, whether Ste20p and Ste50p are regulated by Sho1p or have independent inputs to Ste11p (as shown) is not known. The question marks at the head of filamentation pathway indicate that the initial sensing components have not been identified. Likewise, the question marks at the end of the HOG pathway indicate that a transcription factor target of Hog1p has not been identified. The role of Ste50p in filamentation has not been investigated.



mating pathway (in fact, more than one component—see below), namely the MAPKKK Ste11p. The MAPK Hog1p, and its MAPKK, Pbs2p, are regulated by two independent osmosensors, which lead to activation of distinct MAPKKs. One osmosensor is a membrane-bound protein complex (Sln1p-Ypd1p-Ssk1p), related to bacterial two-component systems, that activates a pair of redundant MAPKKs (Ssk2p and Ssk22p) by a phospho-relay mechanism. The second osmosensor is a presumptive membrane protein, Sho1p. Sho1p binds to Pbs2p, but, until recently the identity of a MAPKKK that might function in this osmosensing branch, if indeed one existed, was unknown. Genetic studies identified Ste11p as the missing MAPKKK (Posas and Saito 1997). Ste11p can phosphorylate Pbs2p and that phosphorylation is Sho1p dependent. The observation that Pbs2p interacts with Sho1p, Ste11p, and Hog1p suggests that, in addition to its catalytic activity, Pbs2p may also serve as a scaffold, although the existence of a multiprotein complex containing these proteins has not been demonstrated.

MAPKs as specificity factors: Hog1p

As the foregoing discussion shows, there is considerable sharing of components by the mating, filamentation, and osmosensing HOG pathways. Notably, all three pathways use Ste11p as a MAPKKK. Nonetheless, no cross-activation or cross talk among the pathways has been observed. Treatment of cells with mating pheromone does not activate either the filamentation or HOG pathways (Madhani et al. 1997; Posas and Saito 1997), and activation of the HOG pathway does not activate the mating pathway (Posas and Saito 1997).

To investigate the mechanism(s) that limits or precludes cross talk between MAPK signaling pathways, O'Rourke and Herskowitz (1998) sought mutants in which high osmolarity induced the mating pathway. This effort identified two genes, *PBS2* and *HOG1*, that is, the genes that encode the MAPKK and MAPK of the

HOG pathway. The cross talk observed is robust: In these mutants, high osmolarity induces transcription of a mating-pathway reporter, *FUS1*, to a level comparable with that seen on pheromone stimulation, although the kinetics of the osmolarity-stimulated induction are slower. Moreover, high osmolarity induces morphological alterations (shmoos) characteristic of pheromone-treated cells and restores partial mating competence to strains defective for G-protein function or for the scaffolding protein Ste5p. To investigate which functions within the HOG pathway and the mating pathway are required for this cross talk, O'Rourke and Herskowitz (1998) constructed double mutants lacking Hog1p and known components of either the HOG or mating pathways. These studies revealed that cross talk requires the Sho1p branch, but not the Sln1p branch, of the HOG pathway. That is, cross talk requires the branch of the HOG pathway that includes Ste11p. Within the mating pathway, cross talk does not require the receptors, the G proteins, or the scaffolding protein Ste5p, but does require all other components.

How does Hog1p function to prevent cross talk? As discussed below, a part of the mechanism that ensures specificity in mating pathway signaling lies in a function of Fus3p, the MAPK that functions in that pathway, that is distinct from its catalytic activity. In the case of Hog1p, in contrast, O'Rourke and Herskowitz (1998) find that mutants with altered active site residues exhibit cross talk, implying that it is the kinase activity per se that prevents promiscuous signaling. They suggest that Sho1p, the presumed receptor for an osmolarity signal, is the relevant target of Hog1p with respect to preventing cross talk. The idea is that phosphorylation of Sho1p by Hog1p is part of the mechanism by which the HOG pathway is down-regulated. Hence, in wild-type cells, activation of the Sho1p branch is only transient and inappropriate activation of the mating pathway does not occur. In *hog1* mutants, however, stimulation of this Sho1p branch is chronic, leading to cross-activation of the mat-

ing pathway. The idea that the duration of MAPK activity may affect the physiological outcome is reminiscent of the proposed mechanism by which cultured PC12 cells exhibit different responses when the same MAPK is activated by different receptor-ligand interactions. Epidermal growth factor causes transient activation of MAPK, which results in proliferation, whereas nerve growth factor causes sustained activation, which results in differentiation (Marshall 1995).

O'Rourke and Herskowitz (1998) report two additional results that have implications for pathway connections and specificity. First, they show that the HOG and mating pathways share more components than previously appreciated. Specifically, the mating pathway components Ste20p and Ste50p (a protein of unknown biochemical function that influences activity of Ste11p) are required for operation of the Sho1p branch of the HOG pathway. Thus, these two pathways share not only Ste11p but also known regulators of Ste11p. Because the filamentation pathway also requires Ste11p and Ste20p (Ste50p has not been tested), there is ample opportunity for communication among these three pathways. Perhaps in wild-type cells there are physiological conditions under which such communication is important. For example, for cell fusion to occur during mating, the cell wall must be restructured and plasma membrane fusion must occur. Hence, there is the risk of cell lysis, so it may be important to coordinate osmolarity and pheromone signals. Second, their work points to a new connection between the HOG and filamentation pathways: Sho1p is necessary for pseudohyphal development. Perhaps Sho1p is a receptor for multiple signals, some of which lead to activation of the HOG pathway, and some of which lead to activation of the filamentation pathway. In any event, the finding that the HOG, mating, and filamentation pathways share a number of components not only intensifies the question of how pathway specificity is maintained, but also suggests that there is the potential for an intricate network of interconnections at the headwaters of these pathways.

MAPKs as specificity factors: Fus3p

The second instance in which a MAPK activity contributes to pathway specificity is provided by the mating pathway MAPK, Fus3p. This MAPK has a biochemical activity, distinct from its catalytic activity, that ensures that activation of the mating pathway does not simultaneously activate the filamentation pathway. This conclusion emerged from efforts to identify the MAPK that functions in the filamentation pathway. As noted already, the mating and filamentation pathways share a number of components, including the MAPKKK Ste11p and the MAPKK Ste7p, but a MAPK that functions in the filamentation pathway had not been identified. In fact, the absence of either MAPK known to be activated by Ste7p, namely Fus3p and Kss1p, does not block activation of the filamentation pathway as measured by pseudohyphal development in diploids. There are at least two possible resolutions to this puzzle of the missing MAPK.

The first is that the relevant MAPK remains to be identified. The second is that Fus3p or Kss1p in fact has a role in the filamentation pathway, but that this role has been unrecognized. The idea is as follows. Because the potential involvement of MAPKs in the filamentation pathway has been investigated by use of deletion mutants, the relevant MAPK would have escaped detection if it had positive and negative activities of approximately equal strength that regulate signaling. In such a case, its absence would have little effect on the filamentation process. Madhani et al. (1997) and Cook et al. (1997) report experiments that support this second explanation and specifically argue that Kss1p is the MAPK for the filamentation pathway. Madhani et al. (1997) go on to argue that Fus3p function is dedicated to the mating pathway, in contrast to the earlier view that held that Fus3p and Kss1p were redundant MAPKs, either of which could function in the mating pathway. Again, the exclusive analysis of deletion mutants precluded the realization that Fus3p had two biochemical functions, a protein kinase catalytic activity required for signaling in the mating pathway and a second, specificity activity.

The possibility that Kss1p could be the MAPK for filamentation was first suggested by the observation that the absence of Kss1p led to a defect in invasive growth in haploids, although there was no apparent defect in pseudohyphal development in diploids. Nonetheless, this result suggests a positive role for this MAPK in the filamentation pathway. A negative role for this MAPK is implied by the finding that deletion of *KSS1* restores pseudohyphal development to diploids that are deleted for *STE7*. More compelling support for the idea that Kss1p has both positive and negative functions comes from the isolation of filamentation-defective and hyperfilamentation Kss1p mutants. The filamentation-defective mutants have amino acid substitutions in residues known to be important for kinase catalytic activity. In contrast, hyperfilamentation mutants have normal kinase activity but are defective for binding to Ste12p, a transcription factor required for filamentation and known to be a target of Kss1p. Together, these experiments lead to the following model. In the absence of an appropriate filamentation signal, Kss1p binds to Ste12p and prevents it from activating transcription of genes required for filamentation. On receipt of the filamentation signal, Kss1p becomes phosphorylated by Ste7p, which relieves its inhibitory activity on Ste12p. Release from Kss1p inhibitory activity is sufficient to allow Ste12p to promote transcription of the target genes and allows a filamentation response. However, it is likely that the Kss1p kinase catalytic activity stimulates an even more robust filamentation response.

If Kss1p is the MAPK for the filamentation pathway, as the above experiments argue, is it also redundant with Fus3p function in the mating pathway? In cells lacking Fus3p, and therefore relying on Kss1p for mating pathway signaling, activation of the mating pathway also leads to activation of the filamentation pathway (Madhani et al. 1997). In wild-type cells this does not happen, so there must be a mechanism that precludes

activation of the filamentation pathway when the mating pathway is stimulated. Madhani et al. (1997) asked whether Fus3p might be the protein that prevents such cross-activation. They found that cells expressing both a catalytically inactive form of Fus3p and wild-type Kss1p could not activate the mating pathway. This result implies that the two MAPKs are not functionally redundant in the mating pathway and further implies that each is dedicated to function in a specific pathway, Kss1p to the filamentation pathway and Fus3p to the mating pathway. Madhani et al. (1997) suggest that Fus3p functions as a specificity factor by preventing Kss1p from interacting with mating pathway specific components, such as the scaffolding protein Ste5p.

The notion of pathway specificity implies, ultimately, that the MAPK activates the correct genetic program. How is this achieved by Fus3p and Kss1p, given that they share Ste12p as a target and that this transcription factor is required to execute both mating and filamentation programs? Part of the answer is that Ste12p participates in a combinatorial regulatory strategy. Ste12p functions in the mating pathway as either a homodimer or a heterodimer with Mcm1p (for review, see Johnson 1995). These forms of Ste12p can bind to the promoters of mating pathway inducible genes, but not those of filamentation pathway inducible genes. The form of Ste12p that participates in the filamentation pathway is a heterodimer with Tec1p; this heterodimer binds to the promoters of filamentation pathway inducible genes, but not mating pathway inducible genes (Madhani and Fink 1997). Although this insight provides a molecular explanation for how different genetic programs are activated by the mating and filamentation pathways, it raises new specificity questions as well. By what mechanism does Fus3p lead to activation of Ste12p homodimer and of Ste12p-Mcm1p heterodimers but not Ste12p-Tec1p heterodimers? Is Ste5p, the scaffolding protein required in the mating pathway but not the filamentation pathway, responsible for this differential effect, or are other proteins involved?

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References

- Cook, J.G., L. Bardwell, and J. Thorner. 1997. Inhibitory and activating functions for MAPK Kss1 in the *S. cerevisiae* filamentous-growth signalling pathway. *Nature* **390**: 85–88.
- Herskowitz, I. 1995. MAP kinase pathways in yeast: For mating and more. *Cell* **80**: 187–197.
- Johnson, A.D. 1995. Molecular mechanisms of cell-type determination in budding yeast. *Curr. Opin. Genet. Dev.* **5**: 552–558.
- Leberer, E., D.Y. Thomas, and M. Whiteway. 1997. Pheromone signalling and polarized morphogenesis in yeast. *Curr. Opin. Genet. Dev.* **7**: 59–66.
- Levin, D.E. and B. Errede. 1995. The proliferation of MAP kinase signaling pathways in yeast. *Curr. Opin. Cell Biol.* **7**: 197–202.
- Madhani, H.D. and G.R. Fink. 1997. Combinatorial control required for the specificity of yeast MAPK signaling. *Science* **275**: 1314–1317.
- . 1998. The riddle of MAP kinase specificity. *Trends Genet.* **14**: 151–155.
- Madhani, H.D., C.A. Styles, and G.R. Fink. 1997. MAP kinases with distinct inhibitory functions impart signaling specificity during yeast differentiation. *Cell* **91**: 673–684.
- Marshall, C.J. 1995. Specificity of receptor tyrosine kinase signaling: Transient versus sustained extracellular signal-related kinase activation. *Cell* **80**: 179–185.
- O'Rourke, S.M. and I. Herskowitz. 1998. The Hog1 MAPK prevents cross talk between the HOG and pheromone response MAPK pathways in *Saccharomyces cerevisiae*. *Genes & Dev.* **12**: (this issue).
- Posas, F. and H. Saito. 1997. Osmotic activation of the HOG MAPK pathway via Ste11p MAPKKK: Scaffold role of Pbs2p MAPKK. *Science* **276**: 1702–1705.
- Robinson, M.J. and M.H. Cobb. 1997. Mitogen-activated protein kinase pathways. *Curr. Opin. Cell Biol.* **9**: 180–186.