Signal transduction by MAP kinase cascades in budding yeast
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Budding yeast contain at least four distinct MAPK (mitogen activated protein kinase) cascades that transduce a variety of intracellular signals: mating-pheromone response, pseudohyphal/invasive growth, cell wall integrity, and high osmolarity adaptation. Although each MAPK cascade contains a conserved set of three protein kinases, the upstream activation mechanisms for these cascades are diverse, including a trimeric G protein, monomeric small G proteins, and a prokaryotic-like two-component system. Recently, it became apparent that there is extensive sharing of signaling elements among the MAPK pathways; however, little undesirable cross-talk occurs between various cascades. The formation of multi-protein signaling complexes is probably centrally important for this insulation of individual MAPK cascades.

Introduction

MAPK (mitogen activated protein kinase) cascades are modular signaling units composed of three protein kinases: a MAPKKK (MAP kinase kinase kinase) activates a cognate MAPKK (MAP kinase) by phosphorylating specific serine/threonine residues; the MAPKK in turn activates a specific MAPK by phosphorylating specific tyrosine and threonine residues [1,2]. MAPK cascades are found both in higher and lower eukaryotic organisms, including yeasts, plants, and mammals [3]. The upstream mechanisms that activate MAPK cascades are diverse and in many cases remain unclear. An activated MAPK phosphorylates specific effector molecules, which include nuclear transcription factors as well as cytosolic proteins. The tandem arrangement of three kinases in each MAPK cascade serves several important purposes. First, a three kinase cascade amplifies the input signal, because one activated kinase at one level may phosphorylate and activate many kinase molecules at the next level. Second, this arrangement confers an acutely sigmoidal responsiveness (hypersensitivity) to signaling pathways: MAPK will not be easily activated by weak signals but when prodded by a stimulus above threshold it will be activated decisively [4]. Third, this arrangement allows integration of distinct upstream signals.

Each organism has multiple distinct MAPK cascades that transduce different signals. For example, budding yeast (Saccharomyces cerevisiae) has at least four different MAPK cascades: the mating-pheromone pathway; the pseudohyphal/invasive growth pathway; the protein kinase C (PKC) pathway; and the osmoregulatory pathway. Recently, it has become apparent that a common element can be used in more than one signal transduction pathways (for example, in the case of the Ste11 MAPKKK, as many as three pathways); however, cross-talk between pathways is averted, probably because of the formation of multi-component signaling complexes. In this article, we review these and other recent findings in the yeast MAPK cascades.

Mating-pheromone pathway

The yeast mating-pheromone response is one of the best characterized signal transduction pathways in eukaryotic organisms. Most of the components involved in this pathway, from the transmembrane pheromone receptors to the nuclear transcription activators and cell cycle regulators, have been identified and characterized [5–7]. The receptors for the α and a mating factors (Ste2 and Ste3, respectively) interact and activate a heterotrimeric G protein, which is composed of Gα (Gpa1), Gβ (Ste4), and Gγ (Ste18) subunits (see Figure 1a). When activated, the dissociated Gβ–Gγ complex transduces signals through Ste20 (a protein kinase of the PAK [p21-activated protein kinase] family) and Ste5 (a scaffold protein) to the mating-pheromone MAPK cascade. Ste5 forms the core of a signaling complex that contains the Ste11 MAPKKK, the MAPKK Ste7, and the Fus3/Kss1 MAPKs [8–11]. Activated Fus3/Kss1 MAPKs then induce the transcription of specific genes by activating the transcription factor Ste12 [12]. Activated Fus3 (but not Kss1) also arrests the cell cycle at the G1/S transition by activating the inhibitor of the Cdc28–Cln kinase, Far1 [13,14].

Major uncertainties remain concerning the roles of the Gβ–Gγ complex, Ste5, and Ste20 in the activation of the mating-pheromone MAPK cascade. In addition to acting as a scaffold, Ste5 is also an active element in transducing the mating-pheromone signal. The interaction between Gβ protein and Ste5 seems essential for signal transduction because Ste5 mutants that cannot bind Gβ are unable to activate the mating-pheromone MAPK cascade, even though such Ste5 mutant proteins still bind all three kinases in the mating-pheromone MAPK cascade ([15,16*]; E. Elion, personal communication).
The Gβ–Ste5 interaction may be a prerequisite for Ste5–Ste5 oligomerization, which occurs during normal signal transduction [16•,17•]. It is not known, however, whether and how the Ste5 oligomerization leads to activation of the mating-pheromone MAPK cascade.

The small GTP-binding protein Cdc42 was shown to interact directly with Ste20 and mutant cells lacking functional Cdc42 or Cdc24—a GDP/GTP exchange factor for Cdc42—are defective in mating, suggesting that Cdc42 is an upstream regulator of the mating-pheromone MAPK cascade [18,19]. Ste20 mutants that no longer bind Cdc42, however, are fully capable of activating the mating-pheromone MAPK cascade, indicating that the pheromone-induced activation of Ste20 is mediated by another factor, perhaps the Gβ–Gγ complex [20•,21•]. The role of Cdc42 in mating is probably to localize Ste20 at shmoo tips in order to facilitate cell–cell adhesion during conjugation. This role for Ste20–Cdc42 during conjugation is most likely mediated by the SH3-domain protein Bem1, which interacts with Ste5, Ste20 and actin [22,23].

**Pseudohyphal/invasive growth pathway**

Diploid yeast cells undergo a dimorphic transition to pseudohyphal (filamentous) growth when starved of nitrogen [24]. In an apparently related phenomenon, haploid cells grow invasively on rich media [25]. Both pseudohyphal development and invasive growth require a subset of the signaling components found in the mating-pheromone pathway (i.e. Ste20, Ste11, Ste7, and Ste12) (see Figure 1b). The upstream mechanism that activates Ras2 is unknown. Activated Kss1 (MAPK) regulates the transcription factors Ste12 and Tec1. Although Ste12 is common to the two signaling pathways, a Tec1–Ste12 complex induces genes specific to the pseudohyphal/invasive growth pathway.

Dominantly active Ras2 (Ras2V19) or Cdc42 (Cdc42V12) induces both filamentous growth and expression of the filamentous-growth-specific reporter gene FG(TyA)::LacZ but not the mating pathway reporter gene FUS1::LacZ [27•]. In contrast, only FUS1::LacZ, but not FG(TyA)::LacZ, is induced by the mating pheromone. Thus, even though the expression of both reporter genes is dependent on Ste20, Ste11, Ste7, and Ste12, there is no cross-talk be-
between the two signaling pathways. Ras2 seems to activate Ste20 through Cdc42 but the upstream mechanism that activates Ras2 is not known. Filamentous growth requires the 14-3-3 proteins Bmh1 and Bmh2, which most likely modify Ste20 to allow signal transmission from Ras2/Cdc42 to downstream components [28]. In contrast, Bmh1 and Bmh2 are not essential for the mating pheromone MAPK cascade signaling.

Although the mating-pheromone pathway and the pseudohyphal/invasive growth pathway share several constituents, the two pathways appear to use different MAPKs. Recent reports suggest that the Kss1 MAPK regulates mainly filamentation, partly by inhibiting Dig1 and Dig2 (negative regulators of invasive growth) [31,32]. Interestingly, however, both Fus3 and Kss1 also have kinase-independent activities that inhibit invasive growth [29,30]. Indeed, fus3 kss1 double mutants grow invasively, indicating that their inhibitory function is as important as their positive roles. As previously shown, Kss1 can mediate mating-pheromone signaling in the absence of Fus3 [33]. Under such conditions, however, there is erroneous cross-talk in which mating pheromone also activates filamentation-specific gene expression [29]. Perhaps a key to this (loss of) mutual insulation is the tethering protein Ste5. If Ste5 binds Fus3 preferentially over Kss1, then signals emanating from the pheromone receptor will be channeled preferentially to a complex containing Fus3 but not Kss1. In the absence of Fus3, Kss1 may have an access to Ste5, causing the observed cross-talk. In contrast, 14-3-3 proteins (Bmh1/2) may route the signal from Ras2 to Ste11 that is not complexed with Ste5.

The pathogenic fungus *Candida albicans* can also switch between unicellular and filamentous forms. This morphological switch is governed by a MAPK cascade homologous to the *S. cerevisiae* pseudohyphal pathway [34,35]. The finding that nonfilamentous *Candida* mutants are avirulent may have important clinical implications [36].

**Protein kinase C pathway**

Yeast Pkc1 is a homolog of mammalian already defined PKC and regulates a MAPK cascade composed of Bck1 (MAPKKK), Mkk1/2 (MAPKKs) and Mpk1 (MAPK) (see Figure 2) [37]. Hypotonic shock and heat shock are known to activate the PKC pathway [38,39]. Mutants in the PKC signaling cascade undergo cell lysis because of a deficiency in cell wall construction that is exacerbated at high temperatures. Mutants of Rho1 (a small GTP-binding protein) display a cell lysis defect similar to that of mutants defective in the PKC pathway. Furthermore, expression of an activated allele of *PKC1* or overexpression of wild-type *PKC1* suppresses the cell lysis defect of *rho1* mutants, suggesting

![Figure 2](image-url)
that Rho1 has a role in the PKC pathway. It was shown recently that GTP-bound Rho1 associates with Pkc1 and confers upon Pkc1 the ability to be stimulated by the co-factor phosphatidylserine. Rho1 is known to be regulated by Rom1/2 (GDP/GTP exchange factors specific to Rho1), Sac7 (a GTPase-activating protein for Rho1), and Tor2 (a phosphatidylinositol kinase homolog and an activator of Rom2). Thus, these molecules may also have roles in the PKC pathway. Rho1, when bound to GTP, is also a positive regulator of 1,3-β-D-glucan synthase (GS). GS is a multi-enzyme complex that catalyzes the synthesis of 1,3-β-linked glucan, a major structural component of the yeast cell wall. Interestingly, expression of Fks2, a component of GS complex, is controlled by the PKC pathway. Thus, GTP-bound Rho1 stimulates cell wall construction directly by activating GS and indirectly by stimulating the PKC pathway.

Mutants of the HCS77 gene exhibit similar phenotypes as PKC pathway mutants and are partially suppressed by overexpression of Pkc1. Furthermore, hcs77 mutants are defective in heat shock induction of Mpk1 activity, suggesting that Hcs77 acts upstream of the PKC MAPK cascade. The PKC pathway is also partially dependent on the Cdc28–Cln1/2 kinase. Thus, the PKC pathway, which promotes bud emergence and cell surface growth, may be activated by the Cdc28–Cln1/2 kinase at the G1/S transition and by Hcs77 upon heat shock or hypo-osmotic shock. The phosphatidylinositol-4-kinase, Stt4, may also be involved in the PKC pathway but its exact role is not clear.

The PKC pathway is also activated by mating pheromones. This apparent cross-talk, however, is an indirect effect of cell wall re-organization induced by the mating-pheromone pathway. The two MAPK cascades are actually well insulated. For instance, under normal conditions, the MAPKK in the mating pathway (Ste7) cannot functionally substitute for the MAPKK in the PKC pathway (Mkk1/2). A Ste7 mutant (Ste7P368) with elevated activity, however, can suppress defects in the PKC MAPK pathway if Ste5 is absent, suggesting that Ste5 is important.

Figure 3

Osmoregulatory signaling pathway. Yeast have two independent osmosensors, Sho1 and Sin1. The Sho1 osmosensor contains four transmembrane segments and a cytoplasmic SH3 domain that binds to the amino terminus of Pbs2 MAPKK. Activation of Pbs2 by Sho1 requires the Ste11 MAPKK, a kinase that is also used in the mating-pheromone pathway and the pseudohyphal/invasive growth pathway. The second osmosensor, Sin1, is homologous to prokaryotic two-component signal transducers and contains two transmembrane segments, a cytoplasmic histidine kinase domain and the phospho-accepting receiver domain. Sin1 is a part of a multistep phospho-relay composed of three proteins (Sin1, Ypd1 and Ssk1) that regulates the osmoregulatory MAPK cascade. Under high osmotic stress conditions, Sin1 histidine kinase is inactivated resulting in accumulation of unphosphorylated Ssk1, which activates the Ssk2/22 MAPKKs, which in turn activate Pbs2. Thus, Pbs2 integrates signals from two independent osmosensors. Activated Pbs2 then phosphorylates the Hog1 MAPK, which induces a set of osmoregulatory responses, including glycerol synthesis.
for preventing undesirable cross-talk between the two pathways [50].

**Osmoregulatory pathway**

The fourth major yeast MAPK cascade regulates adaptation to hyperosmolarity (see Figure 3). Because a major outcome of the activation of the osmoregulatory MAPK pathway is elevated glycerol synthesis, this pathway is frequently referred to as the HOG (high osmolarity glycerol response) pathway [51,52]. The HOG pathway is essential for survival in high osmolarity environments [51,53]. In yeast, extracellular hyperosmolarity is detected by one of two transmembrane osmosensors, Sln1 and Sho1 [54,55]. Although the two osmosensors are not structurally related, both activate the same MAPK, Hog1. Sln1—a homolog of prokaryotic two-component signal transducers—contains two transmembrane segments, a cytoplasmic histidine kinase domain, and a receiver domain [56]. Sln1 transmits signals to the redundant Ssk2 and Ssk22 MAPKKKs, via the Sln1±Ypd1±Ssk1 multi-step phosphorelay system [54,55,57,58]. Ssk1 is also homologous to the receiver domains of prokaryotic two-component signal transducers [54]. The phospho-relay system is initiated by the auto-phosphorylation of Sln1±His576 (see Figure 4). This phosphate is then sequentially transferred to Sln1–Asp1144, then to Ypd1–His64, and finally to Ssk1–Asp554. This four-step phospho-relay reaction has been reconstituted in vitro using purified recombinant proteins [57**]. The end product of the phospho-relay is aspartate-phosphorylated Ssk1. Under normal osmotic conditions, active Sln1 histidine kinase most likely maintains Ssk1 in the phosphorylated state whereas, at high osmotic conditions, the Sln1 histidine kinase is inhibited, leading to the accumulation of unphosphorylated Ypd1 and Ssk1. Unphosphorylated Ssk1 then activates the redundant Ssk2 and Ssk22 MAPKKKs, which in turn phosphorylate and activate the Pbs2 MAPKK, leading to Hog1 MAPK activation [51,55,59].

Activated alleles of the *SLN1* gene *nrp2* encode mutant proteins with a high histidine kinase activity that, when combined with a disruption mutation of the second osmosensor Sho1, cause an osmosensitive phenotype [60]. Sln1*nrp2* mutant proteins may be locked in the phosphorylated state and thus cannot activate the HOG MAPK cascade. Interestingly, *nrp2* mutants have another phenotype, namely hyper-expression of genes regulated by the transcription factor Mcm1. This latter phenotype is independent of the presence or absence of either Ssk1 or the Hog1 MAPK, suggesting that Sln1 may have two distinct and independent roles: regulation of the Hog1

![Multistep phospho-relay reaction in the yeast two-component osmosensor.](current-opinion-in-microbiology-figure-4.png)

Multistep phospho-relay reaction in the yeast two-component osmosensor. (a) Under normal extracellular osmotic conditions, Sln1 autophosphorylates a histidine (H) residue near its histidine kinase domain. (b) This phosphate is then sequentially transferred to the Sln1 carboxy-terminal receiver domain. (c) to a histidine residue in Ypd1, and (d) finally to an aspartic acid (D) in the Ssk1 receiver domain. Under high osmotic stress conditions, Sln1 histidine kinase is inactivated, resulting in accumulation of unphosphorylated Ssk1, which activates the Ssk2/Ssk22 MAPKKKs (see Figure 3). P, phosphorylation.
MAPK and activation of the Mcm1 transcription factor [60,61].

It is not known how changes in extracellular osmolarity regulates the Sln1 cytoplasmic histidine kinase. Increased extracellular osmolarity causes the outflow of water through the plasma membrane, shrinkage of the cytoplasmic volume, and a separation of the plasma membrane and the cell wall (plasmolysis). It is thus possible that the Sln1 extracellular region somehow monitors the distance of the plasma membrane and the cell wall, perhaps by making specific contact with a cell wall component. Another possibility is that the shrinkage of cell volume causes change in the plasma membrane itself, perhaps because of decreased membrane tension, such changes perhaps being detected by Sln1.

Sho1, the second osmosensor, has four predicted transmembrane segments and a carboxy-terminal cytoplasmic region containing an SH3 domain [55]. An SH3 domain interacts with a target protein containing a proline-rich motif [62]. The physiologically relevant target of the Sho1 SH3 domain is Pbs2. A mutant Pbs2 that has a single amino acid substitution (Pro96→Ser) within a proline-rich sequence (KPLPPLPV) fails to interact with Sho1 and is unable to transduce a signal from the Sho1 osmosensor. The same Pbs2 mutant, however, is fully capable of transducing signals from the Sln1 osmosensor [55]. Although Sho1 interacts directly with the Pbs2 MAPKK, the activation of Pbs2 requires the Ste11 MAPKKK [57**]. Thus, constitutively active Ste11 activates both the mating and osmoregulatory MAPK cascades. Despite these findings, however, the two pathways are normally well insulated from each other: mating phosphor-erates activate only the mating pathway, and osmotic stress activates only the HOG pathway [57**]. As previously mentioned, Ste5 is a probable determinant in the signaling specificitv. Furthermore, Pbs2 may also play an important role in determining specificity. As Pbs2 interacts with Sho1, Ste11, and Hog1, it may also have a scaffold-like function [57**].

**Protein phosphatases that regulate MAPK cascades**

In addition to the positive regulatory elements discussed above, MAPK cascades are regulated negatively by a variety of protein phosphatases. In the osmoregulatory pathway, the protein tyrosine phosphatase Ptp2 (and to a lesser extent Ptp3) downregulates the Hog1 MAPK [63]. A type 2C protein serine/threonine phosphatase, Ptc1, has also been implicated in downregulating the osmoregulatory pathway but its exact target is unknown [54,64]. In the mating-pheromone pathway, Ptp3 (and to a lesser extent Ptp2) is responsible for regulating the tyrosine phosphorylation level of the Fus3 MAPK [65]. Furthermore, a dual-specific (i.e. specific to both tyrosine phosphate and threonine/serine phosphate) phosphatase Mgs3 is induced following pheromone stimulation to facilitate the recovery of the pathway to the pre-stimulation state [65,66].

**Conclusions**

A comparison of the four MAPK cascades in *S. cerevisiae* illustrates two important principles that are applicable to other organisms including mammals. First, there exist amazingly diverse ways to activate MAPK cascades; activation of the first kinase in the cascade (MAPKKK) may require a protein kinase (such as Ste20 or Pkc1), or the interaction with another protein (for example Ssk1). Furthermore, upstream elements may involve trimeric and monomeric G proteins, two-component sensors, cyclin-dependent kinases, and transmembrane sensors (such as Hcs77 and Sho1). Second, though a common signaling component may be shared by more than one pathway, there is little, if any, cross-talk between the pathways. For example, the Ste11 MAPKKK is involved in three different pathways; mating-pheromone, pseudohyphal/invasive, and osmoregulatory. Formation of multiprotein complexes (signalosomes) is the probable mechanism to ensure the specificity of each signaling pathway but the way such a complex is formed may be unique to each signaling pathway.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- **of special interest**
- **of outstanding interest**

This paper, as with Inouye main (C177S or C177S C180S) cannot rescue the mating defects of Ste5 is essential for its function because mutations of this do-

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Data in this paper and [29*] suggest that the Fus3 MAPK mainly regulates mating whereas the Kss1 MAPK regulates filamentous and invasive growth. In addition to their kinase-dependent activities, Fus3 and Kss1 have distinct kinase-independent inhibitory functions: inactive Kss1 interacts with and inhibits Ste12, whereas both inactive and active Fus3 prevent invasive growth.


The authors of this paper describe the direct involvement of the Rh1 GT-

Pase in Pkc1 activation. The GTP-bound form of Rho1 confers on Pkc1 the ability to be activated by the cofactor phosphatidylinerine. This provides a po-

ential mechanism to regulate the activity of the PKC pathway by cytokinesecretion and membrane reorganization.

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See annotation [45].


This paper and that of Drigonová et al., 1996 [44] demonstrated that the small GTPase RHOK is a regulatory subunit of the 1,3-β-glucan synthase complex. 1,3-β-linked glucan is a major structural component of the yeast cell wall and RHOK is localized predominantly at sites of cell wall remodeling.


This paper presents evidence that the protein kinase (PKC) pathway promotes bud emergence and organizes surface growth, as well as being activated by Cdc28-Cln1/Cln2 at the G1/S transition. This paper also suggests that a type I transmembrane protein, Hsc77, may monitor the integrity of the cell surface, and activate PKC pathway upon heat shock.


This paper also presents evidence that the PKC pathway is activated by the Cdc28-Cln1/Cln2 kinase at the G1/S transition. These authors further propose that the increased production of diacylglycerol mediated by the Cdc28-dependent phosphatidylinositol-specific phospholipase C may be responsible for Pkc1 activation.


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This paper demonstrates that a multistep phospho-relay mechanism regulates the phosphorylation state of the Ssk1 receiver domain protein. Phosphotransfer reactions are examined both in vivo and in vitro.


This paper demonstrated that Ste11 MAPKK is essential for the activation of the Pbs2 MAPKK by the Sho1 osmosensor. In spite of the sharing of Ste11 by the mating and osmoregulatory pathways, there is no cross-talk between them. It is proposed that Pbs2 might function as a scaffold protein in the osmoregulatory pathway because Pbs2 binds Sho1, Ste11, and Hog1.


