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How can yeast cells decide between three activated MAP kinase pathways? A model approach

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ABSTRACT

In yeast (*Saccharomyces cerevisiae*), the regulation of three MAP kinase pathways responding to pheromones (Fus3 pathway), carbon/nitrogen starvation (Kss1 pathway), and high osmolarity/osmotic stress (Hog1 pathway) is the subject of intensive research. We were interested in the question how yeast cells would respond when more than one of the MAP kinase pathways are activated simultaneously. Here, we give a brief overview over the regulatory mechanisms of the yeast MAP kinase pathways and investigate a kinetic model based on presently known molecular interactions and feedbacks within and between the three mitogen-activated protein kinases (MAPK) pathways. When two pathways are activated simultaneously with the osmotic stress response as one of them, the model predicts that the osmotic stress response (Hog1 pathway) is turned on first. The same is true when all three pathways are activated at the same time. When testing simultaneous stimulations by low nitrogen and pheromones through the Kss1 and Fus3 pathways, respectively, the low nitrogen response dominates over the pheromone response. Due to its autocatalytic activation mechanism, the pheromone response (Fus3 pathway) shows typical sigmoid response kinetics and excitability. In the presence of a small but sufficient amount of activated Fus3, a stimulation by pheromones will lead to a rapid self-amplification of the pheromone response. This 'excitability' appears to be a feature of the pheromone pathway that has specific biological significance.

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1. Introduction

Cells must respond to changes in their environment—either to biological signals such as pheromones, hormones, neurotransmitters or to chemical/physical signals such as nutrient concentrations, osmolarity changes, oxidative stress, temperature changes or radiation. They perceive these signals by specific receptors or sensing mechanisms which then elicit intracellular signaling cascades, information processing and a more or less specific set of cellular responses. Cells have to decide between different responses if more than one signal is perceived at a time—similar to neuronal systems (Uchida et al., 2006). The mechanisms of decision-making within cells are not well understood, however.

In eukaryotic cells different groups (modules) of mitogen-activated protein kinases (MAPK) play a decisive role in intracellular signaling. MAPKs are involved in the control of cell processes such as proliferation, differentiation, stress reactions, memory, apoptosis, and aging (Kyriakis and Avruch, 2001; Cowan and Storey, 2003). They are well conserved in all eukaryotic organisms

from yeast to mammals and consist of different modules each of which is engaged in the signal transmission and processing of a different set of signals (Roux and Blenis, 2004). MAP kinase pathways consist of 3–4 layers of upstream protein kinases, which form an amplification cascade. The different layers—from top to bottom—are termed MAPKKKK (Ste20), MAPKKK (MEKK, Ste 11), MAPKK (MEK, Ste 7) and the central MAP kinases or modules which consist of a number of isoforms. They control the activity of various target proteins such as stress proteins, downstream protein kinases or transcription factors. Upstream and downstream components of these modules are partially identical and can principally cross-react with components of other modules (cross-talk) which raise questions with respect to the separation of the pathways and the mechanisms of decision-making if more than one pathway is activated at a time (Blagosklonny, 2003).

One answer to the cross-talk problem is the existence of scaffold proteins that bind related MAPK components and put them into a sequential spatial orientation for a correct intracellular flux of information (Helmbrecht et al., 2000; Schwartz and Madhani, 2004). Scaffold proteins thus insulate specific signaling pathways against each other. In case of simultaneous activation of different pathways, competitive interactions between the pathways (mutual inhibition) may be of particular importance for the

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dominance of a specific signal pathway. The survival value of a signal may be expressed in terms of the amplification capacity of the respective signaling MAPK module and by the strength of (mostly inhibitory) interactions with other modules.

In order to test these assumptions we developed a minimal model based on presently known positive and negative feedback loops within and negative interactions across the MAPK pathways in yeast (*S. cerevisiae*) and explored model decisions when the MAPK pathways were stimulated simultaneously. We chose signal pathways that lead either to mating, to filamentous invasive growth or to high osmolarity responses (Schwartz and Madhani, 2004). Central components of the three pathways are the MAP kinases Fus3 for the mating response, Kss1 for filamentous invasive growth and Hog1 (high osmolarity glycerol) for high osmolarity and other stress responses.

The model suggests answers to a number of questions—for example: (i) why does the Fus3 pathway contain several positive feedback loops, whereas the Hog1 pathway has none; (ii) why has the filamentous growth pathway apparently no feedback regulation, and how do these different properties influence the decision of cells, if two or three of these pathways were activated simultaneously or sequentially?

First, we will give a short overview over the spatial arrangement of the MAPK components, their positive and negative feedback systems as well as their mutual interactions. We will then simplify the regulatory networks and describe them by means of differential equations. Since many of the rate constants, amplification factors, concentrations of components, and other parameters of the interacting components are not exactly known, this modeling approach is necessarily qualitative in nature, but nevertheless may still allow an estimation of some fundamental features of the systems. Modeling may also allow to estimate the influence of various parameters such as signal strength, amplification capacity, inhibitory action or temporal sequence of signals on the decision between the three modules.

Several models of the three MAPK systems in yeast have been published recently following different approaches (Somsen et al., 2002; Kofahl and Klipp, 2004; Klipp et al., 2005; Schaber et al., 2006; Hao et al., 2007a; McClean et al., 2007; Zou et al., 2008). We have included positive feedback loops in the pheromone pathway

(see description below), which have not been considered in previous models, but which can affect the relative dominance of the MAPK pathways when they are activated simultaneously or sequentially. When constructing our model we have reduced the number of rate equations and adjustable parameters/rate constants by grouping together processes and feedback regulatory steps (Fig. 2). We think that the resulting 'minimal model' can make predictions about essential properties of the MAPK network, which can be further explored or tested by more detailed models and/or experiments.

2. The three MAP kinase pathways

The yeast signal pathways to pheromone response, filamentous growth and high osmolarity response have been reviewed recently (Gustin et al., 1998; Schwartz and Madhani, 2004; Bardwell, 2006; Dard and Peter, 2006). The different signal pathways have two levels of upstream protein kinases in common: the MAPKKKK (Ste20) and the MAPKKK (Ste11). Two of the pathways (pheromone response and filamentous growth) also share the same MAPKK (Ste7). In the following section, we will briefly summarize the components of the three pathways, their control mechanisms as well as their mutual interactions.

2.1. The pheromone response pathway

The pheromone pathway (Fig. 1 left) is initiated by pheromone molecules (in this case by α -factor), which are released by haploid α -cells. The pheromone binds to its receptor (Ste2) on haploid α -cells. The activated receptor interacts with heterotrimeric G-proteins (Hao et al., 2007a), consisting of α , β and γ subunits and induces the exchange of GDP against GTP in the α -subunit and a subsequent dissociation of the α - and β - γ subunits. Via Cdc42 the membrane-bound β / γ subunit activates the p21 activated kinase (PAK) termed Ste20. Cdc42 is a membrane-bound Rho-type GTPase essential for cell division and for the control of cellular development during mating and invasive growth. It also activates another PAK (Cla4) which has the potential to act as a negative

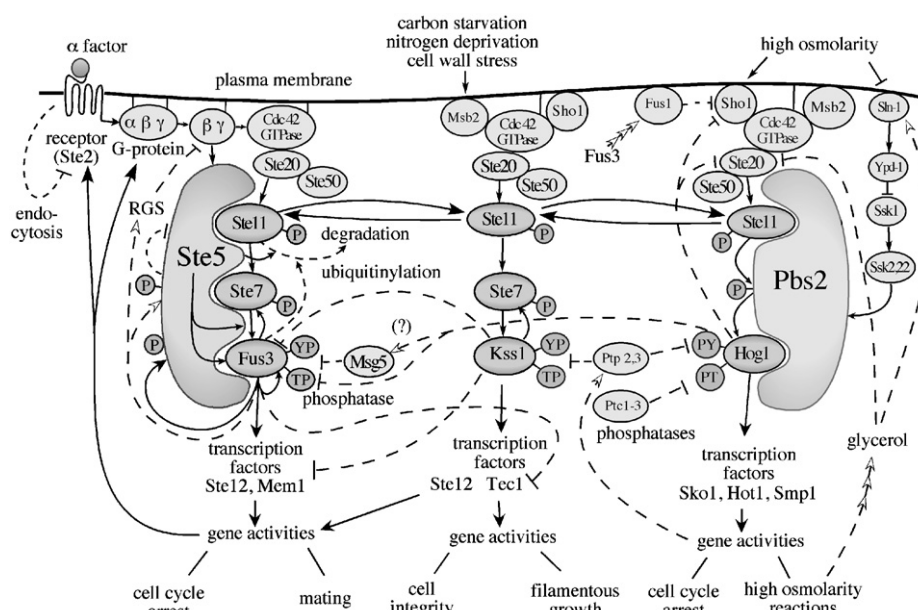


Fig. 1. MAPK regulation in the mating response (left), invasive filamentous growth (middle) and high osmolarity response (right) in yeast. See text and Tables 1 and 2 for description.

Table 1

Positive and negative regulatory mechanisms and constitutive positive and negative factors in the pheromone response pathway of yeast.

<i>Positive regulatory mechanisms</i>	
(i)	G β (Ste4) causes a conformational change of the scaffold protein Ste5 and thus enhances the Ste7–Fus3 contact (Flatauer et al., 2005).
(ii)	Ste5 induces an allosteric activation of Fus3 autophosphorylation (Bhattacharyya et al., 2006).
(iii)	Ste5 phosphorylation by the pheromone response MAPK-cascade, particularly by Fus3 leads to increased membrane recruitment and stabilization against degradation of Ste5 (Flotho et al., 2004).
(iv)	Fus3 induces synthesis of α -factor receptor and G-protein (Yi et al., 2003).
<i>Negative regulatory mechanisms</i>	
(v)	Ste5 phosphorylation at T287 by mono-phosphorylated Fus3 leads to Ste5 inhibition (Bhattacharyya et al., 2006).
(vi)	Fus3-dependent activation of a membrane-bound regulator of G protein signalling (RGS) leads to increased GTPase activity at the G-protein and thus to a decrease of its activity (Garrison et al., 1999; Hao et al., 2007a).
(vii)	Ste5-dependent increase of Ste11 ubiquitinylation and subsequent degradation results in an attenuation of signal transduction (Dard and Peter, 2006).
(viii)	Fus3-dependent phosphorylation of Ste11 also results in its ubiquitinylation and subsequent degradation (Esch and Errede, 2002).
(ix)	Endocytosis of the α -factor receptor after binding of α -factor (Yi et al., 2003).
(x)	Active Ste12 induces the expression of an extracellular endoprotease (Bar1), which is secreted and degrades the α -factor (Ballensiefen and Schmitt, 1997).
(xi)	An attenuation of the pheromone pathway is achieved by a pheromone-dependent ubiquitinylation of Ste7 (Wang and Dohlman, 2002).
<i>Constitutive factors</i>	
(xii)	Constitutive concentrations of the pathway components.
(xiii)	Dephosphorylation of kinases by phosphatases: particularly of Fus3 by Msg 5 and Ptp2 and 3.
(xiv)	Degradation rates of the pathway components.

regulator of the mating pathway (Heinrich et al., 2007) (not shown).

The β/γ subunit also recruits the scaffold protein Ste5 to the membrane, such that the membrane-associated kinase (Ste20) bound to Cdc42 and the adaptor protein Ste50 can phosphorylate and thus activate Ste11. Ste11 binds to the scaffold protein and activates Ste7, which in turn activates Fus3—and also Kss1—the kinases that are phosphorylated at a tyrosine (Y) and threonine (T) residue. Fus3 then activates transcription factors such as Ste12 and Mcm1 by inactivating their inhibitors. Homodimers of Ste12 and heterodimers of Ste12 and Mcm1 initiate gene activities necessary for mating. Fus3 also activates Far1 that causes cell cycle arrest (not shown). Recent studies have shown that many MAP kinases control gene activities not only by activating the transcription factors by phosphorylation but also by binding to transcription factors at promoter sites (Pokholok et al., 2006).

All activation steps of the MAPK-cascade result in signal amplification. The exact degree of these amplifications is not known. The pheromone signal is further amplified by positive feedback or feedforward processes which are built in this pathway (Fig. 1, Table 1). Their quantitative effects have also not been determined. In the model, we therefore chose identical amplification kinetics.

The kinetics of the pheromone response pathway has been subject to several experimental and modeling studies (Kofahl and Klipp, 2004; Schaber et al., 2006; McClean et al., 2007). During a later phase of this response or during a longer-lasting exposure to the pheromone negative feedback mechanisms attenuate the pathway (Fig. 1, Table 1). In addition to the amplification and later down regulation of the MAPK-cascade pathway by the above mentioned mechanisms some constitutive factors such as the concentrations and degradation rates of the pathway components as well as the dephosphorylation rate of kinases by phosphatases

play a role (Fig. 1, Table 1). Altogether, these factors and the signal strength determine the amplitude of the pheromone response and the robustness of this pathway in competition with other pathways.

2.2. Filamentous invasive growth pathway

A transition from vegetative to pseudohyphal growth is induced on one side in diploid cells on solid medium by limited nitrogen and on the other side in haploid cells in response to carbon starvation or alcohol. In the pseudohyphal (filamentous) invasive growth mode chains of elongated cells remain attached and form filaments (Maleri et al., 2004)—perhaps with the aim to grow into a better environment.

Two transmembrane proteins (Msb2 and Sho1) are the upstream regulators of the filamentous invasive growth pathway (Cullen et al., 2004, Fig. 1 middle). Again, the Cdc42/Ste20 complex together with the adaptor protein Ste50 are involved in the subsequent phosphorylation of Ste11. Ste11 phosphorylates Ste7 which then activates the MAP kinase Kss1. Ste5 can activate Kss1 by generating a pool of Ste11, while additional scaffolding by Ste5 is needed for Fus3 activation (Andersson et al., 2004). No specific scaffold protein seems to be involved in the filamentous growth pathway. Persistent activation by constitutive Ste7 promotes Kss1-dependent invasive growth—but not Fus3-dependent mating (Maleri et al., 2004). Kss1 also phosphorylates Ste7 and forms a stable complex with it (Bardwell et al., 1996). Kss1 in turn activates transcription factors such as Ste12, Tec1, and others which are involved in filamentous invasive growth and cell integrity by inactivating their inhibitors (Schwartz and Madhani, 2004).

In contrast to the pheromone response pathway this pathway shows—to our knowledge—neither positive nor negative feedbacks. An RNA-binding protein (MPTS) seems to inhibit Ste7 and Tec1 when no signals are transmitted along this pathway, perhaps depending on the phosphorylation of Ste7 and seemingly aimed at preventing activation of this pathway by random molecular fluctuations (Prinz et al., 2007). The constitutive factors are principally identical to those listed in Table 1 for the pheromone response pathway. Some findings suggest that invasive growth is not a single unified state but can be initiated by different signals including cyclic AMP and the protein kinase A (Breitkreutz et al., 2003; Maleri et al., 2004).

2.3. High osmolarity pathway

This pathway (Fig. 1 right) is activated by high external osmolarity and other stressors and eventually leads to cellular adaptation, for example by activation of ion transport and glycerol synthesis which prevent dehydration of the cell by high external osmolarity. Components of this pathway and their interrelations have been reviewed (Hohmann, 2002). Two starting points are presently known: both consist of transmembrane proteins, Sho1 and Msb2 on one side, Sln1 (a histidine kinase) on the other. The Sho1 starting point involves the membrane-associated complex of Cdc42 and Ste20 together with the adaptor protein Ste50 (Tatebayashi et al., 2006; Wu et al., 2006). This complex is activated by high external osmolarities (0.5–1.0 M NaCl) and leads to phosphorylation of Ste11. Ste11 is associated with the scaffold protein and protein kinase Pbs2, which apparently is also recruited to the plasma membrane. Ste11 phosphorylates Pbs2, which in turn phosphorylates the MAP kinase Hog1 (high osmolarity glycerol 1).

The Sln1 starting point initiates another signal cascade. Under iso-osmotic conditions, Sln1 acts through a relay of phosphopro-

teins to hold two highly related MAPKKK isoforms (Ssk2 and Ssk22) in the inhibited state. Moderate increase of external osmolarity (0.1–0.4 M NaCl) somehow blocks Sln1 function thereby relieving inhibition of the two MAPKKs (Maeda et al., 1994). These kinases in turn phosphorylate and activate the MAPKK Pbs2 (Westfall and Thorner, 2006). Ssk2 is also involved in the actin cytoskeleton re-polarization after osmotic shock (Bettinger and Amberg, 2007).

Pbs2 activates Hog1 (associated with HSP90 and Cdc37p). Hog1 activates transcription factors (Sko1, Hot1, Smp1) involved in high osmolarity responses (Hohmann, 2002). It also stimulates directly a Na/H antiporter and a potassium channel in the plasma membrane as an early response to higher external NaCl osmolarity (Proft and Struhl, 2004; Proft et al., 2005).

Hog1, furthermore, targets a deacetylase to osmostress-responsive genes and may thus activate these genes for a longer period of time (De Nadal et al., 2004). Again, this cascade contains several amplification steps, while positive feedback systems are not known. Several negative feedback circuits, however, attenuate the pathway particularly when an adapted state is reached: The transcription factor Hot1 activates genes that code for enzymes involved in glycerol synthesis such as glycerol-3 phosphate dehydrogenase (GPD) which leads to higher glycerol levels in the cell. High glycerol in turn inhibits the Sho1-dependent pathway and the Sln1 pathway. Hog1 furthermore activates the transcription factor Sko1 that enhances the expression of tyrosin phosphatases (Ptp2, 3) which dephosphorylate and thus inactivate Hog1. Hog1 inhibits Ste20 and Sho1 at the very beginning of the pathway. The constitutive factors are similar to those mentioned above (Table 1).

2.4. Interactions between the pathways

Mutual negative interactions between the three pathways determine the decision of the cell to a great extent when exposed to two or three signals simultaneously (Fig. 1): (i) Fus3 prevents Kss1 from activating invasive growth genes by two mechanisms: by lowering the magnitude and duration of Kss1 activation and by phosphorylating Tec1—a transcription factor of invasive growth genes—resulting in Tec1's ubiquitinylation and degradation (Bao et al., 2004); (ii) Fus3 influences the Hog1 pathway negatively via expression of Fus1, a membrane protein that inhibits Sho1 (Nelson et al., 2004); (iii) Hog1 inhibits Fus3 (Hall et al., 1996) possibly by activating the phosphatase Msg5; (iv) Hog1 also inhibits Kss1 by increasing the amount of phosphatases (Ptp2, 3) which dephosphorylate not only Hog1 itself (negative feedback) but also Kss1; (v) Feedback phosphorylation of Ste7 by Kss1 specifies an invasive growth response through selective activation of Kss1 and filamentation-specific gene expression. This occurs because mating-specific gene expression is suppressed by the feedback phosphorylation status of Ste7 (Maleri et al., 2004); (vi) Ste11 MAPKKK molecules are components of each of the three pathways. It is unclear, however, whether a binding competition exists among the pathways or an overflow of the Ste11 molecules from an activated pathway to another pathway.

Remarkably, a single amino acid (E756) in the Ste5 scaffold, which is located in the Ste7 binding domain is critical for the activation of the mating response. A point mutation of E756 causes a shift from activation of Fus3 to activation of Kss1 in response to mating pheromone. Apparently, this mutation causes a dissociation of phosphorylated Ste7 from the scaffold, leading to pheromone hyperactivation of Kss1 and a severe defect in the activation of Fus3 (Schwartz and Madhani, 2004). Such mutations or variability in binding properties of scaffold proteins are, however, constitutive factors of the pathways.

Recent data indicate that scaffold proteins can also recruit phosphatases to the MAPK-cascade and thus limit the signal pathway. The yeast adaptor protein Nbp2p for example is required for osmotic stress and mediates association to the type 2C phosphatase Ptc1p to Pbs2p which thus leads to inactivation of Hog1 (Mapes and Ota, 2004). In the mating pathway of yeast, the phosphatase Msg5 acts as a negative regulator by inactivating Fus3—with no effect on Kss1p. This specificity may be due to binding of Fus3 to Ste5 (Dard and Peter, 2006).

A recently published model proposes that specificity of the pheromone pathway is due to a simultaneous activation of Ste5 and Fus3 resulting in a coincidence detector (or molecular AND gate); allowing Fus3 to ignore leaking signals most of the time (Bardwell, 2006).

3. Modeling

3.1. Construction of the model

A problem in the construction of a quantitative model is that many of the rate constants and kinetic parameters/binding constants in the MAPK pathways are still not known, although estimates and experimental values for certain rate constants have been implemented in recent models (Kofahl and Klipp, 2004; Klipp et al., 2005; Schaber et al., 2006; Zou et al., 2008). We were interested how the positive and negative (inter and intra) pathway interactions influence the realization of each pathway in the presence of simultaneous activation of other pathways. Based on the regulatory mechanisms of the MAPK pathways described above and summarized in Fig. 1, we constructed a

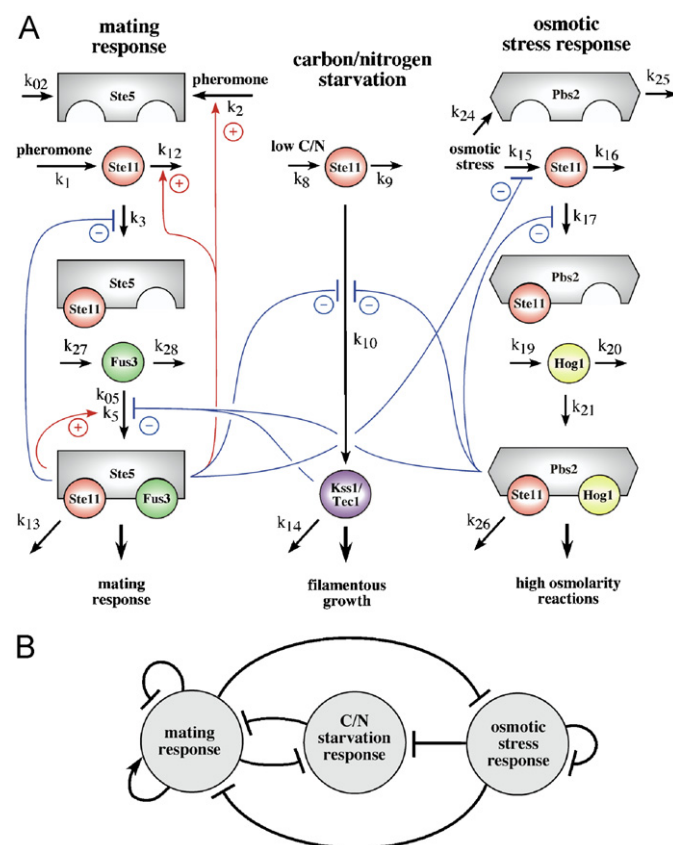


Fig. 2. A. Kinetic model of the three MAPK pathways based on the scheme shown in Fig. 1. See Eqs. (1)–(18) and Table 3 for details. B. Global inhibition and activation pattern in the model.

minimal semi-quantitative model (Fig. 2A), which incorporates the essential processes, as well as the feedback structures of the three different MAPK pathways (Fig. 2B). In order to keep the model as simple as possible, we considered that the enzymes catalyzing the various processes were not saturated reducing Michaelis–Menten kinetics to first- and second-order reactions.

The model consists of 12 kinetic variables with 27 rate constants:

$$\frac{d[\text{Ste11}_{\text{mat}}]}{dt} = k_1 - (k_3[\text{Ste5}] + k_{12})[\text{Ste11}_{\text{mat}}] + k_4[\text{Ste11}_{\text{mat}} \cdot \text{Ste5}] \quad (1)$$

$$\frac{d[\text{Ste5}]}{dt} = k_{02} + k_2 - k_3[\text{Ste5}][\text{Ste11}_{\text{mat}}] + k_4[\text{Ste11}_{\text{mat}} \cdot \text{Ste5}] \quad (2)$$

$$\begin{aligned} \frac{d[\text{Ste11}_{\text{mat}} \cdot \text{Ste5}]}{dt} &= k_3[\text{Ste5}][\text{Ste11}_{\text{mat}}] \\ &\quad - (k_4 + k_5[\text{Fus3}])[\text{Ste11}_{\text{mat}} \cdot \text{Ste5}] \\ &\quad + k_6[\text{Ste11}_{\text{mat}} \cdot \text{Ste5} \cdot \text{Fus3}] \end{aligned} \quad (3)$$

$$\begin{aligned} \frac{d[\text{Fus3}]}{dt} &= k_{27} - k_5[\text{Ste11}_{\text{mat}} \cdot \text{Ste5}][\text{Fus3}] \\ &\quad + k_6[\text{Ste11}_{\text{mat}} \cdot \text{Ste5} \cdot \text{Fus3}] - k_{28}[\text{Fus3}] \end{aligned} \quad (4)$$

$$\begin{aligned} \frac{d[\text{Ste11}_{\text{mat}} \cdot \text{Ste5} \cdot \text{Fus3}]}{dt} &= k_{05} + k_5[\text{Fus3}][\text{Ste11}_{\text{mat}} \cdot \text{Ste5}] \\ &\quad - (k_6 + k_{13})[\text{Ste11}_{\text{mat}} \cdot \text{Ste5} \cdot \text{Fus3}] \end{aligned} \quad (5)$$

$$\frac{d[\text{Ste11}_{\text{lowC/N}}]}{dt} = k_8 - (k_9 + k_{10})[\text{Ste11}_{\text{lowC/N}}] \quad (6)$$

$$\frac{d[\text{Kss1/Tec1}]}{dt} = k_{10}[\text{Ste11}_{\text{lowC/N}}] - k_{14}[\text{Kss1/Tec1}] \quad (7)$$

$$\begin{aligned} \frac{d[\text{Ste11}_{\text{os}}]}{dt} &= k_{15} - k_{16}[\text{Ste11}_{\text{os}}] - k_{17}[\text{Ste11}_{\text{os}}][\text{Pbs2}] \\ &\quad + k_{18}[\text{Ste11}_{\text{os}} \cdot \text{Pbs2}] \end{aligned} \quad (8)$$

$$\begin{aligned} \frac{d[\text{Pbs2}]}{dt} &= k_{24} - k_{17}[\text{Ste11}_{\text{os}}][\text{Pbs2}] \\ &\quad + k_{18}[\text{Ste11}_{\text{os}} \cdot \text{Pbs2}] - k_{25}[\text{Pbs2}] \end{aligned} \quad (9)$$

$$\begin{aligned} \frac{d[\text{Ste11}_{\text{os}} \cdot \text{Pbs2}]}{dt} &= k_{17}[\text{Ste11}_{\text{os}}][\text{Pbs2}] \\ &\quad - k_{18}[\text{Ste11}_{\text{os}} \cdot \text{Pbs2}] \\ &\quad - k_{21}[\text{Ste11}_{\text{os}} \cdot \text{Pbs2}][\text{Hog1}] \\ &\quad + k_{22}[\text{Ste11}_{\text{os}} \cdot \text{Pbs2} \cdot \text{Hog1}] \end{aligned} \quad (10)$$

$$\begin{aligned} \frac{d[\text{Hog1}]}{dt} &= k_{19} - k_{20}[\text{Hog1}] - k_{21}[\text{Ste11}_{\text{os}} \cdot \text{Pbs2}][\text{Hog1}] \\ &\quad + k_{22}[\text{Ste11}_{\text{os}} \cdot \text{Pbs2} \cdot \text{Hog1}] \end{aligned} \quad (11)$$

$$\begin{aligned} \frac{d[\text{Ste11}_{\text{os}} \cdot \text{Pbs2} \cdot \text{Hog1}]}{dt} &= k_{21}[\text{Ste11}_{\text{os}} \cdot \text{Pbs2}][\text{Hog1}] \\ &\quad - (k_{22} + k_{26})[\text{Ste11}_{\text{os}} \cdot \text{Pbs2} \cdot \text{Hog1}] \end{aligned} \quad (12)$$

Parameter and rate constant values are shown in Table 3.

Because Ste11 molecules (together with Ste50, Ste20, Cdc20, as well as the scaffold proteins Ste5 or Pbs2; Fig. 1) are recruited to receptor-specific sites at the plasma membrane by pheromone/mating (mat), carbon starvation/nitrogen deprivation (lowC/N) or osmotic stress (os) induced signals, we have labeled Ste11 species according to their recruiting environments, i.e., as Ste11_{mat}, Ste11_{lowC/N}, and Ste11_{os}. However, an exchange or competition between these Ste11 species, as indicated in Fig. 1, has not been

implemented into the model, because no data are available on such dynamics.

3.2. Activation and inhibition kinetics

In the mating response (Fig. 2A) the ternary complex Ste11 · Ste5 · Fus3 activates its own production (rate constant k_5), the degradation of Ste11 (rate constant k_{12}), and the induction of Ste5 (rate constant k_2). The activation of these three processes are described as

$$k_i = k_i^0 [\text{Ste11}_{\text{os}} \cdot \text{Ste5} \cdot \text{Fus3}]^{\alpha} \quad (13)$$

with $0 \leq \alpha \leq 1$ and $i \in \{2, 5, 12\}$. In order to avoid that the two positive feedbacks from Ste11_{os} · Ste5 · Fus3 to k_2 and k_5 lead to zero reaction rates in the formation of Ste5 when active Ste11_{os} · Ste5 · Fus3 is initially absent, both Ste5 and Ste11_{os} · Ste5 · Fus3 need to be made constitutively as indicated by the two processes with rate constants k_{02} and k_{05} (Fig. 2A). All inhibitions of reactions “j” are formulated as

$$k_j = \frac{k_j^0}{1 + (a_{jX}[X])^9} \quad (14)$$

where k_j is the rate constant of process “j” during inhibition and k_j^0 is the rate constant in the absence of inhibitor X. For the sake of simplicity we assume that all a_{jX} values are equal (Table 3) leading to equal inhibitory strengths. In case two compounds Y and Z are inhibiting the same process ‘l’ (such as inhibition of k_{05} by Kss1/Tec1 and Ste11 · Pbs2 · Hog1, Fig. 2A), the following expression is used:

$$k_l = k_l^0 \cdot \frac{1}{1 + (a_{lY}[Y])^9} \cdot \frac{1}{1 + (a_{lZ}[Z])^9} \quad (15)$$

The transcriptional activities of target genes for each pathway are considered to be proportional to the respective active MAPKs, i.e.,

$$\text{Fus 3 activity (mating response)} \sim [\text{Ste11} \cdot \text{Ste5} \cdot \text{Fus3}] \quad (16)$$

$$\text{Kss1 activity (low carbon/nitrogen response)} \sim [\text{Kss1/Tec1}] \quad (17)$$

$$\text{Hog1 activity (osmotic stress response)} \sim [\text{Ste11} \cdot \text{Pbs2} \cdot \text{Hog1}] \quad (18)$$

4. Results

4.1. Single and multiple pathway stimulations

The different behaviors of the model during stimulation of one, two or all three model MAPK pathways are shown in Fig. 3.

Both the mating (Fus3) and the high osmolarity pathway (Hog1) contain negative feedback loops, by which the MAPKs (Ste11 · Ste5 · Fus3, and Ste11 · Pbs2 · Hog1) inhibit their own activation (Tables 1 and 2). Because of these negative feedback loops and due to the MAPK degradation/inhibition reactions with rate constants k_{13} and k_{26} , both Fus 3 and Hog1 activities will eventually return/adapt to zero (or low) levels when each of these two pathways are activated for longer periods of time (Figs. 3A and B).

No negative feedback is implemented in the carbon starvation/nitrogen deprivation (Kss1/Tec1) pathway. Therefore, a constant stimulation of Ste11 by low carbon/nitrogen is predicted by the model to lead to a steady state determined by the rate of activation and deactivation/degradation of Kss1/Tec1 (Fig. 3C) (Schaber et al., 2006).

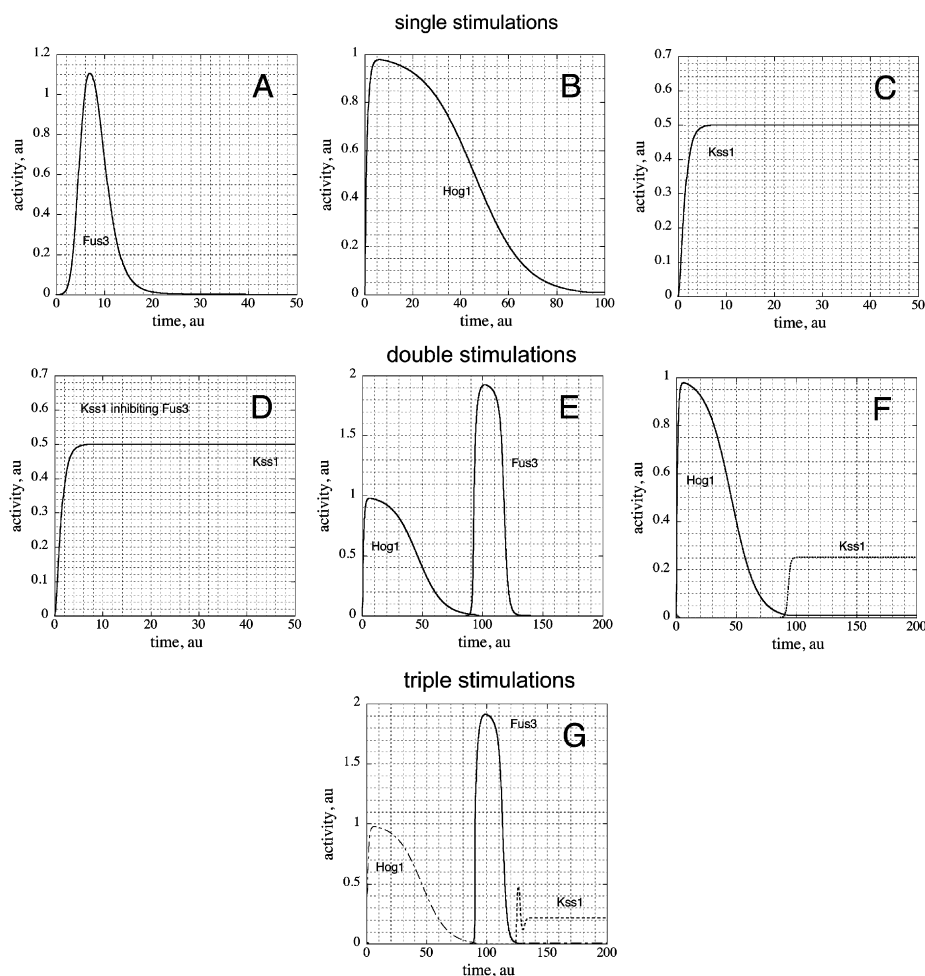


Fig. 3. Model dynamics after stimulation of a single pathway (A–C), of two pathways (D–F) and of all three pathways simultaneously (G). All stimulations occur at time $t = 0$ with all initial concentrations equal to zero.

Table 2

Negative feedback in the high osmolarity glycerol pathway.

- (i) Hog1-dependent higher glycerol concentration inhibits Ste20/Sho1/Pbs2 association and activates Sln1 (Hohmann, 2002).
- (ii) Transcription factor Sko1-dependent synthesis of phosphatases Ptp2, 3 increase dephosphorylation of Hog1-Tyr- $\textcircled{P}$ (Proft et al., 2005).
- (iii) Hog1 inhibits Ste20 (O'Rourke and Herskowitz, 1998).
- (iv) Hog1 inhibits the osmosensor Sho1 by phosphorylation at Ser-166 (Hao et al., 2007b; Mettetal et al., 2008).

When a simultaneous stimulation by low carbon/nitrogen and pheromones is implemented, Ste11 will be up-regulated for each of the two pathways. However, the filamentous growth response will dominate over the mating response (Fig. 3D), because Kss1/Tec1 inhibits the assembly of the Ste11 · Ste5 · Fus3 complex, and Ste11 · Ste5 · Fus3 inhibits its own activation. In addition, the autocatalytic activation of Ste11 · Ste5 · Fus3 by the positive feedbacks to k_5 and k_2 shows a delayed sigmoidal response profile (an induction period¹), which can be inhibited in its early phase by the hyperbolic activation of Kss1 (Fig. 4A). Because Hog1 shows also a hyperbolic initial activation (Fig. 4A), Hog1 will also inhibit Fus3 activation initially. When the activity of Ste11 · Pbs2 · Hog1 is eventually down-regulated by its negative feedback to k_{17} , the

inhibition of k_5 and k_{05} will be relieved such that the autocatalytic production of Ste11 · Ste5 · Fus3 can start when Ste11 · Pbs2 · Hog1 have reached a sufficiently low level (Fig. 3E).

When the Hog1 and Kss1 pathways are stimulated simultaneously, Hog1 dominates over Kss1 due to the inhibition of Kss1 by Hog1 (Fig. 2A). After Hog1 has reached sufficiently low levels due to its autoinhibition (negative feedback) Kss1 will be activated and reach a steady state level which is determined by its activation and deactivation rates as well as by a small residue of Hog1 activity that still inhibits Kss1 (Fig. 3F).

As concluded from the stimulations of the two pathways in Figs. 3D–F one would expect that a simultaneous stimulation of all three pathways would first lead to the activation of Hog1 (because Hog1 is dominating over both Fus3 and Kss1), and then to the activation of Kss1 (because Kss1 dominates over Fus3) keeping Fus3 at low levels. However, the model predicts that Fus3 is activated prior to Kss1. While Hog1 indeed inhibits both Kss1 and Fus3 initially, upon its decline both Fus3 and Kss1 will increase. Due to the autocatalytic (positive feedback) loops in the Fus3 pathway, the presence of active Fus3 may allow for an exponential increase once Hog1 has reached a certain lower threshold. This amplification of Fus3 can occur considerably faster than the Kss1 activation, but requires a sufficient and available amount of Fus3 such that Fus3 amplification can “ignite” (Fig. 3G, Fig. 4B). However, when Fus3 is activated after a simultaneous Hog1 and Kss1 activation, not enough active Fus3 may be formed that is necessary to start an autocatalytic amplification of Fus3. Accord-

¹ The induction period of an autocatalytic system is the time needed to reach its inflection point (see Fus3 kinetics in Fig. 4A).

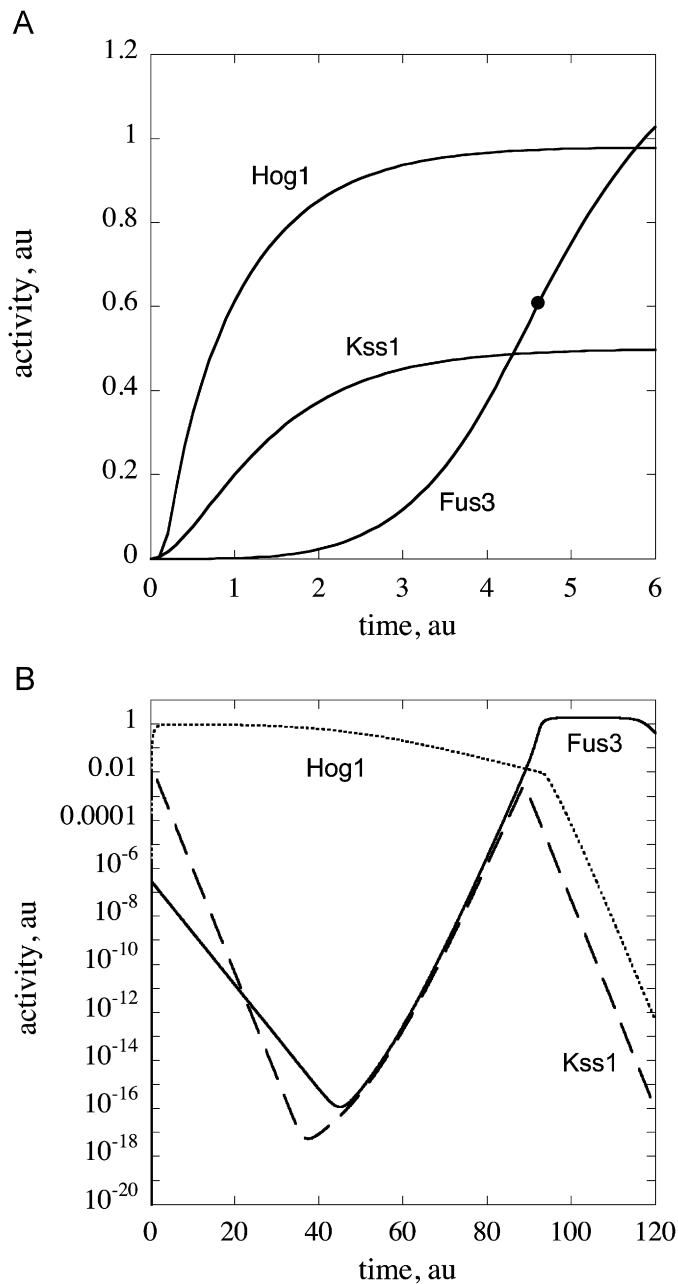


Fig. 4. A. Response kinetics of single stimulations of Hog1, Kss1 and Fus3 pathways. Due to the positive feedback in the Fus3 pathway, Fus3 activity shows a delayed sigmoidal response. The dot indicates the inflection point of the Fus3 response. B. Logarithmic representation of Hog1, Fus3 and Kss1 response in Fig. 3G (simultaneous stimulation). In the presence of sufficient amounts of Fus3, Fus3 is activated by its positive feedback leading to the inhibition of Kss1.

ingly, we observe suppression of Fus3 by Hog1 and Kss1 in the model when the Fus3 pathway is stimulated 80 time units (or less) after both Hog1 and Kss1 have been activated.

4.2. Robustness of the model towards rate constant variations

In order to test the robustness of the model with respect to changes in the rate constants, we increased and decreased all rate constants by two orders of magnitude. The results show that the model is robust with respect to changes in many of the rate constants. In the following we describe the influences of single rate constant changes compared to the “standard” responses as shown in Fig. 3.

Table 3

Rate constant values and initial concentrations.^a

k_1	1.0	k_{12}^0	1.0	k_{21}	10.0
k_2^0	1.0	k_{13}	0.5	k_{23}	1.0
k_3^0	1.0	k_{14}	1.0	k_{24}	1.0
k_4	1.0	k_{15}^0	10.0	k_{25}	1.0
k_5^0	1.0	k_{16}	1.0	k_{26}	1.0
k_7	1.0	k_{17}^0	10.0	k_{27}	1.0
k_8	1.0	k_{18}	1.0	k_{28}	1.0
k_9	1.0	k_{19}	10.0	k_{02}	0.1
k_{10}^0	1.0	k_{20}	1.0	k_{05}^0	0.1

^a Rate constant values are given in arbitrary units. The apparently ‘missing’ rate constants k_6 , k_{11} , and k_{22} were originally assigned to the reverse reactions with respect to k_5^0 (k_5), k_{10} , and k_{21} , respectively, but not used in the irreversible version of the presented model. If not explicitly stated otherwise, initial concentrations are assumed to be zero for all reaction species. For all shown calculations $\alpha = 0.1$ (Eq. (13)), and $a_{jx} = 120$ (Eq. (14)).

When increasing the values of k_{02} , k_1 , k_2^0 , k_3^0 , k_{27} , as well as k_5^0 or k_{05} in the Fus3 pathway (Fig. 2A) 100-fold (relative to the values given in Table 3), no significant changes in the mating response (as shown in Fig. 3A) is observed. However, when lowering the values for these rate constants, except for k_{27} , the Fus3 peak decreases in amplitude and increases in width and will not return to zero values when these rate constants have decreased 10- to –100-fold. When varying k_{27} , we see no significant changes in the Fus3 response neither by increasing nor decreasing its standard value (Table 3) 100-fold. Thus, by choosing k_{02} , k_1 , k_2^0 , k_3^0 and k_5^0/k_{05} values approximately 10-fold higher than those given in Table 3, a robust Fus3 response can be obtained for any 10-fold change of these rate constants. The situation is somewhat reversed when looking at variations in the degradation rate constants k_{12}^0 , k_{13} or k_{28} . A successive increase of k_{12}^0 or k_{28} will first lead to a broadening of the Fus3 pulse and finally to a total loss of the Fus3 pulse leaving the system in a low steady state of Fus3 (Ste11 · Ste5 · Fus3) activity, while a practically unchanged Fus3 response is observed when decreasing k_{12}^0 or k_{28} 100-fold or more. The Fus3 response is quite sensitive to changes in k_{13} . For example, by increasing k_{13} the pulse-width of Fus3 increases dramatically and for (absolute) values greater than 1.0 a.u., the system moves into a steady state where Ste11 · Ste5 · Fus3 steady state values decrease with increasing k_{13} .

The Kss1/Tec1 response (Fig. 3C) shows no adaptation behavior as found for Fus 3 and Hog1 responses (Figs. 3A, B), i.e. during the carbon starvation/nitrogen deprivation response the model predicts that active Kss1 goes into a steady state without returning to its pre-stimulation value. The $[Kss1/Tec1]_{ss}$ steady state is given by the following relationship

$$[Kss1/Tec1]_{ss} = \frac{k_8 k_{10}}{k_9 k_{11} + k_9 k_{14} + k_{10} k_{14}} \quad (19)$$

which shows saturation behavior with increasing k_{10} values.

In the Hog1 pathway, a 100-fold increase of k_{24} increases the amplitude of the standard Hog1 response (Fig. 3B) by approximately 10-fold together with almost a doubling of the pulse-width. This behavior is different from the Fus3 pathway, where the synthesis rate of the scaffold protein (Ste5) does no influence the shape of the Fus3 pulse. Increasing k_{25} narrows the Hog1 pulse and decreases its amplitude, while increasing k_{25} leads to an increase in width, which, when k_{25} reaches zero (all other rate constants as in Table 3), reaches a final pulse-width, which is approximately two times broader (but with the same amplitude) than the reference pulse shown in Fig. 3B. Increasing k_{15} 100-fold does not change the amplitude but increases the pulse-width of the Hog1 response by a factor of two, while increasing k_{16} 100-fold

has the opposite effect, i.e., leads approximately to a doubling of the Hog1 pulse-width, but leave the amplitude practically unchanged. Changes in k_{17} have quantitatively a similar effect as increasing k_{15} , i.e., they lead to an increase in the Hog1 pulse-width, but leaving the amplitude approximately constant. An increase of k_{19} by 100-fold does not change the Hog1 response compared to Fig. 3B, while reducing it by 100-fold decreases the amplitude by one order of magnitude and increases the Hog1 pulse-width by approximately 25%. A corresponding 100-fold increase or decrease of k_{20} or k_{21} does not change the Hog1 response significantly. As observed for the Fus3 and Kss1/Tec1 responses, also the Hog1 response is quite sensitive to changes in k_{26} , i.e., to variations in the degradation/inactivation of the active ternary complex Ste11 · Pbs2 · Hog1.

We also looked at the response sequence of Hog1, Fus3 and Kss1 with respect to rate constant variations when all three pathways are stimulated simultaneously. Although we see the changes in the individual MAPK responses as described above, most of the rate constant variations preserved the activation order as shown in Fig. 3G, except for (100-fold) increases in k_8 or k_{28} where the Fus3 response is suppressed, and for increases in k_{13} or k_{14} in which the Kss1 response is suppressed. For a 100-fold increase in k_{26} the Fus3 response is suppressed, but both Kss1 and Hog1 have significantly reduced amplitudes.

These observations indicate that the MAPK responses by the model are relatively stable against rate constant variations.

5. Discussion

5.1. MAPK pathway dominance by cross-inhibition

We addressed the question what would happen, if all three pathways were triggered simultaneously by the different signals of the same strength—a situation that would not be easy to verify experimentally because of the different numbers of receptors and different receptor-signal affinities of the different pathways. Which pathway would win? There is good *a priori* reason to believe that the high osmolarity pathway would be the dominating one because of its strong suppression of Fus3 and because of its high survival value in the response to endangering stressors. The pheromone pathway, on the other hand, has the highest amplification capacity and inhibits both the filamentous and the high osmolarity pathway. From its biological significance one would assume that this pathway needs to be activated only in the presence of opposite (a or α) mating partners and in the absence of acute stress. Chronic starvation or other long lasting environmental obstacles may favor filamentous invasive growth that may result in the escape from areas of low nutrient contents by growing into a more favorable environment. Another way to cope with detrimental factors is to increase recombination of genetic material—either by sexual recombination (pheromone pathway) or by increasing retrotransposition of transposons such as Ty1 (Conte and Curcio, 2000; Morillon et al., 2000) (adaptive mutagenesis).

Our model shows that both the high osmolarity response and the carbon/nitrogen starvation response dominate over the mating response when simultaneous stimulations of Fus3/Kss1 or Fus3/Hog1 are applied with zero initial concentrations (Figs. 3D, E). These results are similar to those by Zou et al. (Zou et al., 2008), who found in their model that the pheromone and filamentous pathways do not influence the HOG pathway. In our model, the reason for the dominance of the Hog1 and Kss1 pathways over Fus3 is due to an initial delay in the rise of the Fus3 ternary complex (Ste11 · Ste5 · Fus3) which is often observed in autocatalytic kinetics (positive feedback regulation). This delay is

dependent on the constitutive rate constant k_{05} . The lower k_{05} the more pronounced is the induction period with the result that both the Hog1 and Kss1 pathways dominate over Fus3. Alternatively, if k_{05} is relative high, the sigmoid response in Ste11 · Ste5 disappears. Due to the inherent autocatalysis in Fus3 activation, Fus3 then dominates over the Kss1 and Hog1 pathways.

However, even when k_{05} is relatively low Fus3 will be able to inhibit the Kss1 and Hog1 pathways in the presence of relative small amounts of Ste11 · Ste5 · Fus3 that are initially present. The situation is illustrated in Fig. 5, showing that there is an abrupt

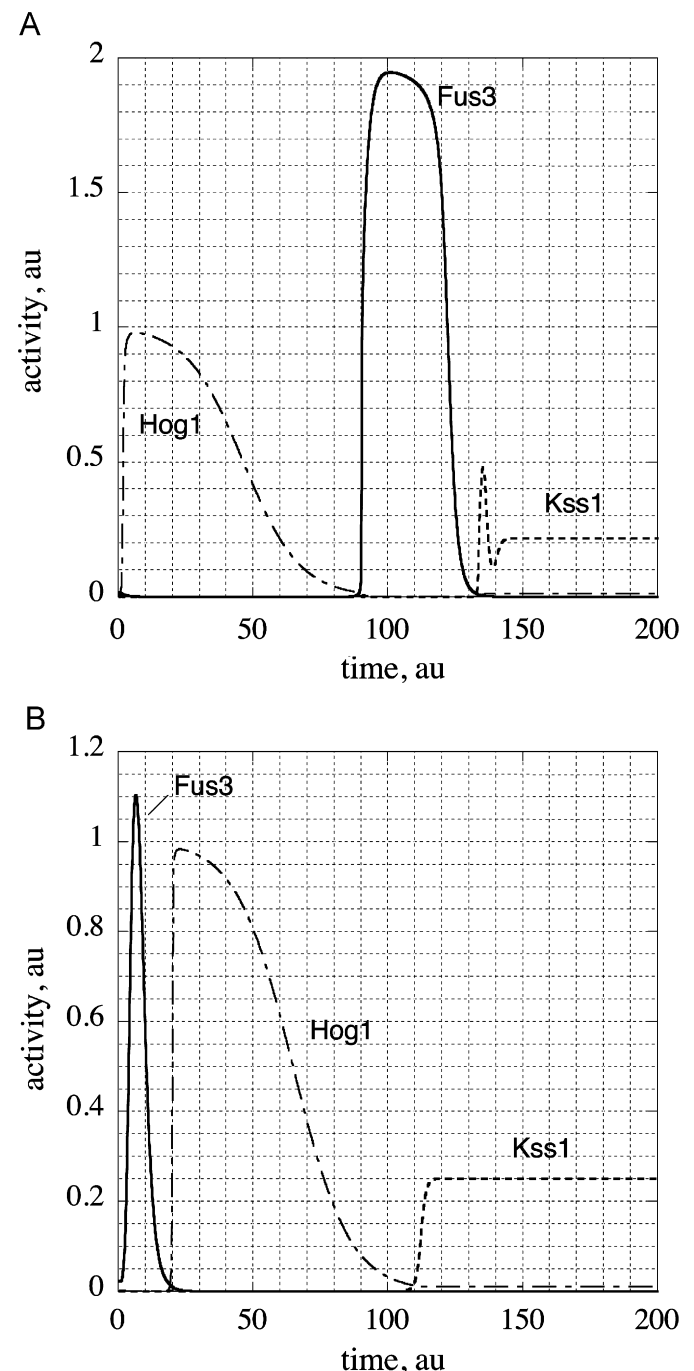


Fig. 5. Stimulation of the three pathways as in Fig. 3G but with small initial amounts of Ste11 · Ste5 · Fus3 present at $t = 0$. (A) $[\text{Ste11} \cdot \text{Ste5} \cdot \text{Fus3}] = 0.02$. The response is practically identical to that shown in Fig. 3 apart from a slightly broader Fus3 peak. (B) $[\text{Ste11} \cdot \text{Ste5} \cdot \text{Fus3}] = 0.03$. The response is dramatically different from Figs. 5A and 3G showing excitability with the mating response coming now before the osmotic stress response and the low nitrogen response.

threshold (≥ 0.03) in the initial amount of active Fus3 (Ste11 · Ste5 · Fus3). Once this threshold is exceeded an excitation of active Fus3 is generated. Small amounts of active Fus3 will thus facilitate/activate a mating response, which will dominate over the other two MAPK pathways. This indicates that the positive feedback loops (autocatalysis) within the Fus3 pathway may play an important role in the decision which of the MAPK pathways are up-regulated during a simultaneous activation.

Another scenario is a temporal sequence in the activation of the different pathways. One would assume that a first high osmolarity signal will probably inhibit the other two pathways until an osmotic steady state is reached. On the other hand, an early and fully amplified pheromone response may suppress temporarily the high osmolarity response or the low nitrogen response. Each of these possibilities are actually observed in the model, but their realization depends on the relative timing of the stimulations and whether residual Ste11 · Ste5 · Fus3 is present or not. Thus, even small variations in environmental conditions may result in different responses of otherwise identical cells. Recent experiments (McClean et al., 2007) allowed to characterize the response of individual yeast cells when the mating response and the osmotic stress response are activated simultaneously. Interestingly, McClean et al. observed that identical yeast cells showed a bistable response, i.e., cells were coexisting, which either showed a mating response or an osmotic stress response, but not both. Such a variability in otherwise identical cells may be of importance for the population (McClean et al., 2007). From our results, we believe that such a bistable response is related to the autocatalytic and inhibitory loops within the MAPK network, which depends on the history of the cells and on small variations of their local environment.

The presence of positive and negative feedback loops may also lead to oscillatory behavior. We have occasionally observed damped oscillations in Hog1 and Kss1 for certain combinations of positive and negative feedback loops and rate constant values (data not shown). However, the significance of such oscillations is presently not clear.

5.2. Biological significance

An important issue is the biological significance of the proposed kinetics for the three MAPK pathways derived from their regulatory mechanisms and interactions. From our model we come to the following suggestions:

- The Fus3 pathway probably reacts to low concentrations of pheromone molecules and therefore needs several (auto-catalytic) amplification steps. The kinetics show a steep and short mating response after a sigmoidal start. The mating response is suppressed by either of the two other pathways, because they are concerned with cellular survival. The Fus3 pathway, may, however, temporarily dominate over Hog1 and Kss1 in the presence of small amounts of Fus3. An immediate and strong activation of this pathway appears to be requested because the opposite mating type cell is available probably only for a short time.
- The Kss1 pathway reacts to a usually long-lasting starvation by a continued, i.e., nonadapted filamentous growth response. The apparent lack of negative feedback regulations in this pathway makes sense, because there is no reason why the organism should adapt to continuous starvation.
- The Hog1 pathway is essential for the adaptation to environmental stress which endangers the survival of the cell. It is therefore necessary to suppress the other pathways in order to enhance the adaptation process. After the osmotic

balance has been established the high osmolarity response is stopped by several negative feedbacks, thus allowing the cell to respond to stimulations of the other MAPK pathways.

It should be mentioned that the suggested model refers only to haploid yeast cells. In nature, budding yeast becomes rapidly diploid after germination of the two mating types. In the diploid state components of the pheromone pathway are repressed such that the cells will only respond to osmotic shock or/and to carbon/nitrogen limitation. Whether our model and the referred experimental findings for the Kss1 and Hog1 pathways also apply to diploid yeast is not known.

6. Computational method

The differential equations were solved numerically by using the FORTRAN subroutine LSODE (Radhakrishnan and Hindmarsh, 1993) interfaced with GNUPLOT using a PERL script (available on request).

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