Signal integration in budding yeast

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Abstract

A complex signalling network governs the response of Saccharomyces cerevisiae to an array of environmental stimuli and stresses. In the present article, we provide an overview of the main signalling system and discuss the mechanisms by which yeast integrates and separates signals from these sources. We apply our classification scheme to a simple semi-quantitative model of the HOG (high-osmolarity glycerol)/FG (filamentous growth)/PH (pheromone) MAPK (mitogen-activated protein kinase) signalling network by perturbing its signal integration mechanisms under combinatorial stimuli of osmotic stress, starvation and pheromone exposure in silico. Our findings include that the Hog1 MAPK might act as a timer for filamentous differentiation, not allowing morphological differentiation before osmo-adaptation is sufficiently completed. We also see that a mutually exclusive decision-making between pheromone and osmo-response might not be taken on the MAPK level and transcriptional regulation of MAPK targets. We conclude that signal integration mechanisms in a wider network including the cell cycle have to be taken into account for which our framework might provide focal points of study.

Introduction

Yeasts have the capability to sense and adapt swiftly to changes in their environment to ensure continued fitness and survival when exposed to external stressors or to initiate a mating programme when brought into contact with pheromones. Most environmental cues are sensed by plasma membrane receptors and relayed via biochemical networks – signalling pathways – to the transcriptome, causing an altered gene expression more suited to the conditions outside the cell. In the present article, we discuss the basic signalling networks and signal integration mechanisms in the budding yeast Saccharomyces cerevisiae and study the impact of some of the mechanisms on signalling in the MAPK (mitogen-activated protein kinase) signalling network.

A complex signalling network governs the response of Saccharomyces cerevisiae to an array of environmental stimuli and stresses. In the present article, we provide an overview of the main signalling system and discuss the mechanisms by which yeast integrates and separates signals from these sources. We apply our classification scheme to a simple semi-quantitative model of the HOG (high-osmolarity glycerol)/FG (filamentous growth)/PH (pheromone) MAPK (mitogen-activated protein kinase) signalling network by perturbing its signal integration mechanisms under combinatorial stimuli of osmotic stress, starvation and pheromone exposure in silico. Our findings include that the Hog1 MAPK might act as a timer for filamentous differentiation, not allowing morphological differentiation before osmo-adaptation is sufficiently completed. We also see that a mutually exclusive decision-making between pheromone and osmo-response might not be taken on the MAPK level and transcriptional regulation of MAPK targets. We conclude that signal integration mechanisms in a wider network including the cell cycle have to be taken into account for which our framework might provide focal points of study.

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Key words: mitogen-activated protein kinase (MAPK), Saccharomyces cerevisiae, signal integration, signal separation.

Abbreviations used: FG, filamentous growth; FRE, filamentous response element; HOG, high-osmolarity glycerol; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MAPKKK, MAPKK kinase; PH, mating pathway; PKA, protein kinase A; PKC, protein kinase C; TOR, target of rapamycin; TORC, TOR complex.

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Notable exceptions are the phosphatases Msg5 and Ptp2, which are both responsible for the deactivation of the PKC pathway’s Slt2 MAPK [2] and the FG/PH network’s Kss1 and Fus3 MAPKs [3]. Whether these phosphatases play a role in signal integration and separation is, however, not known at present.

Other important pathways
Comparably highly connected are also the TOR (target of rapamycin), SNF (sucrose non-fermenting) and PKA (protein kinase A) signalling pathways which are involved in the response to glucose and nitrogen. At the core of the nitrogen-responsive TOR pathway are TORC (TOR complex) 1 and TORC2, with TORC1 being rapamycin-sensitive and implied in the inhibition of the glucose-responsive Snf1 kinase. TORC2, on the other hand, is a potential co-regulator of the Rho1 G-protein upstream of the PKC MAPK cascade [4]. In addition, the TOR pathway shares various transcriptional targets such as Msn2/4 and Fhl1 with the PKA pathway. The PKA pathway itself is both activated by Ras1/2 via an unknown mechanism and by the glucose sensor Gpr1p. Its activation leads to an increased production of cAMP which sets PKA free from a complex
with Bcy1. Upon its release, PKA becomes catalytically active and plays important roles in growth regulation, pseudohyphal growth and filamentous differentiation.

**Signal integration**
In their natural environment, yeasts are often exposed to a complex combination of various stimuli ranging from nutritional and temperature to osmolarity and mating signals. In such situations, multiple pathways can become active and their signals need to be acted upon in an optimal sequence and timing to attain the best adaptation possible. In budding yeast, this is achieved by signal integration and cross-talk processes [5,6].

Signal integration can occur within signalling pathways when multiple pathway branches converge on one target branch or it can describe the phenomenon that signals from different pathways are merged to elicit an adequate response to complex environmental variations. At the same time, signal separation mechanisms are in place to prevent unwanted cross-talk in situations where only one specific part of the signalling system is supposed to respond [7].

Signal integration and separation can thus be best characterized as signalling network properties which cause the network to respond in an optimal way to environmental stimuli.

**Yeast as a model organism**
Basic yeast signalling pathways are phylogenetically preserved in higher eukaryotes. The p38 signalling pathway in human cells regulates the response to hyperosmolality and p38 exhibits high homology with the yeast Hog1 protein [8]. This and other similarities open the opportunity that some of the knowledge and principles around signal integration acquired through research with yeast can be transferred to human cell lines. In humans, faulty cross-talk and signal integration are often associated with diseases such as Alzheimer’s disease [9] and cancer [10].

In the following sections, we discuss the most important mechanisms of signal integration and signal separation. For an overview, see Table 1.

**Dynamic mechanisms of signal integration and signal separation**

**Frequency modulation**
Recent data suggest that yeasts are able to encode information about the extracellular environment in the frequency of stochastic and non-linear phenomena inside the cell [11]. The transcription factor Crz1 translocates to the nucleus in stochastic bursts whose interspike interval is reduced upon external stimulation by calcium. It is believed that transcriptional activation of downstream targets of Crz1 is a result of temporal integration of its nuclear residence.

**Signal duration**
Often, signalling systems can only be affected by certain signals during specific states. In yeast, for instance, cell-cycle arrest by pheromones can only occur in the G1 phase (Figure 2). This is due to the fact that, in haploid budding yeast, Far1p, whose transcription is up-regulated upon pheromone exposure, inhibits the Cln3–Cdc28 dimer which is required for cell-cycle entry into ‘start’. Additionally, phosphorylation of Far1p by the PH pathway MAPK Fus3p enhances the binding of Far1p to the Cln3–Cdc28 complex and thereby increases Far1p’s inhibitory potential.

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**Table 1** | Overview of dynamic and structural mechanisms of signal integration and signal separation

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Signal integration</th>
<th>Signal separation</th>
</tr>
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<tbody>
<tr>
<td>Dynamic</td>
<td>Frequency modulation</td>
<td>Frequency modulation</td>
</tr>
<tr>
<td></td>
<td>Signal duration</td>
<td>Signal duration</td>
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<tr>
<td></td>
<td>Feedback</td>
<td>Feedback</td>
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<tr>
<td></td>
<td>Mutual/hierarchical inhibition</td>
<td>Structural</td>
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<td></td>
<td>Shared components</td>
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<td></td>
<td>Common transcriptional targets</td>
<td>Scaffolding</td>
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<td></td>
<td>Compartmentalization</td>
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</table>

**Figure 2** | Pheromone stimulation can only arrest the cell cycle within G1 phase
For this reason, the duration of pheromone signalling determines whether or not cell-cycle arrest will occur.

**Pheromone**

![Diagram of pheromone signalling pathway]
Feedback
Feedback may help to influence context-dependent signal duration and thereby aid signal integration or separation. For instance, filamentous growth is thought to require constitutive activity of Kss1 [12]. Although Kss1 is cross-activated by the MAPKK Ste7 upon pheromone stimulation, its duration of activity is limited by a negative feedback mediated by Fus3p [13]. This finding suggests that a minimum duration of Kss1 activity might be required to induce filamentous differentiation (Figure 3).

Mutual/hierarchical inhibition
Frequently, it has been hypothesized that mutual inhibition of signalling components could provide an mechanism for signal separation and specificity [14], it has, however, never been demonstrated conclusively in experiments within the S. cerevisiae signalling system to the best of our knowledge. Hierarchical inhibition is exercised by components upstream in the signalling network and is frequent in yeast. It often involves the differential regulation of the transcriptome upon specific stresses. Within the PH/FG pathway, asymmetrical hierarchical inhibition of filamentous response genes by active Fus3p might act as an additional mechanism to prevent accidental filamentous growth upon pheromone exposure.

Structural mechanisms of signal integration and separation
Shared components
Shared components are signalling molecules on which branches of the signalling network converge (see Figure 1). The HOG/FG/PH signalling network include examples such as the MAPKK Pbs2p which integrates the Sho1 and Sln1 branches of the HOG pathway and the Ste11 MAPKKK which integrates and distributes input signals into all three pathways.

Common transcriptional targets
Often, different signalling pathways or networks converge on promoters of key genes, as shown in Figure 4. For instance, the PKA and the FG pathway converge on the promoter of the FLO11 gene [15], which is required for filamentous differentiation.

Sequestration
Sequestration occurs when a signalling component is recruited away from other signalling components to prevent signalling (signal separation) or recruited to other signalling components to enable signalling (signal integration). In budding yeast, this phenomenon, for instance, occurs in the PH pathway where Ste5p and the MAPKs are recruited to sites at the membrane where pheromone is sensed [16].
Scaffolding
Scaffolding can be considered a special case of sequestration. A scaffold protein can additionally act as a signal integrator (Pbs2p), but it prevents signal leakage equally as well as sequestration. Scaffolding may be the main mechanism to isolate the HOG from the PH pathway in budding yeast [17] as shown in Figure 5.

Compartmentalization
Compartmentalization describes the translocation of signalling factors to the nucleus or other cellular compartments to enable or to prevent signalling. Examples in S. cerevisiae include the translocation of the MAPK Hog1p to the nucleus upon its activation by hyperosmotic stress [18]. In the nucleus, Hog1p then activates transcription factors which initiate the transcription of osmo-adaptation genes.

Systematic study of the impact of signal integration mechanisms in the HOG/FG/PH signalling network
We constructed a simple mathematical model (Figure 6 and see the Supplementary online data at http://www.biochemsoctrans.org/bst/038/bst0380000add.htm) of the HOG/FG/PH signalling network and identified five dynamic mechanisms of signal integration and separation – the negative feedback in the HOG [19] and PH [20] pathways, the inhibition of Hog1 activity by active Kss1 [21], the hierarchical inhibition of Tec1 activity on the FRE (filamentous response element) by both active Hog1 [22] and active Fus3 [23] – and two structural mechanisms – the sequestration and scaffolding properties of Pbs2 in the HOG pathway and of Ste5 in the PH pathway. We validated the model by successfully comparing its temporal and qualitative behaviour with available experimental data [24,25] under osmotic stress and pheromone stimulation. For each of these mechanisms, we identified we implemented a mode of perturbation (Table 2) into the model to assess the impact on and the role of a mechanism in the pathway dynamics. The model was then simulated under all combinations of input stresses with modes of perturbations (Figure 7). Overall, we find that the signal integration/separation mechanisms in the HOG/FG/PH network exercise a local influence, i.e. perturbations of signal integration/separation in the PH pathway do not affect osmo-adaptation, and perturbations...
in the HOG pathway have virtually no impact on the pheromone response. To our surprise, this is even true when we allow for increased signalling from the HOG and PH pathways to free Ste11, which, in our model, activates not only Kss1 strongly, but also Hog1 and Fus3 weakly.

However, a strong cross-activation of Kss1 also increases its inhibitory potential on Hog1 activity. In this way, a signal flow from the PH to the HOG pathway can be prevented effectively. On the other hand, Fus3 is shielded by the requirement to bind to Ste5 to become active from excessive activity of free Ste11.

For parallel stimulation of the HOG and the FG pathways, the activity of Tec1 on the FRE increases more slowly than for FG pathway stimulation alone. This effect is even stronger if the strength of the negative feedback in the HOG pathway is reduced or if Kss1 inhibition on Hog1 is weakened. For strong cross-activation of free Kss1 from the HOG pathway and decreased inhibition of Tec1 by Hog1, the effect
is diminished. This suggests that, in the presence of both osmotic stress and nutrient depletion, Hog1 activity might act as a timer, not allowing filamentous differentiation to occur before osmo-adaptation is completed.

The fact that most signal integration and separation mechanisms in the HOG/FG/PH network act locally shows again in the observation that, upon parallel stimulation of both the HOG and the PH pathways, both respond in nearly the same way as when stimulated alone and again, only local mechanisms appear to distort their dynamics. One notable exception is increasing the cross-talk to free Ste11 from the PH pathway. The subsequent Kss1 hyperactivation does not allow Hog1 to become active in the presence of osmotic stress via the previously described inhibitory mechanism.

Co-stimulation of the FG pathway and the PH pathway reveals that Fus3 might also act as a timer for filamentous differentiation. In the presence of active Fus3, Tec1 activity is rising more slowly than under the exclusive nutrient-depletion stress. The timer is, however, disrupted as expected, if the inhibitory effect of active Fus3 on Tec1 activity is reduced and also if cross-talk from the PH pathway is enhanced.

When all three stresses are applied at the same time, the increase of Tec1 activity is even slower because of the fact that now both active Hog1 and active Fus3 are inhibiting Tec1’s activity until the negative-feedback loops in their respective pathways down-regulate their own activity.

Conclusions
After a brief overview of the most important signalling systems in budding yeast, we introduced a new framework of classifying signal integration and separation mechanisms which goes beyond the classical concepts of signalling pathways, cross-talk and specificity. The framework allows us to understand cellular signalling as an integrative interplay between network components and modules rather than a task of individual insulated signalling pathways.

We systematically studied the influence of these mechanisms on a stress- and pheromone-stimulated HOG/FG/PH signalling network and reveal, that although their influence is often local, it is paradoxically Ste5 scaffolding, which is thought to reduce cross-talk from the PH pathway to Kss1 and the HOG pathway, that allows Hog1 to become transiently active under simultaneous stimulation of the HOG and PH pathways. We also show that Hog1 might act as a timer, which prevents filamentous differentiation from taking place before adaptation to osmotic stress has been sufficiently completed.

Additionally, our modelling approach also indicates that a mutually exclusive decision between pheromone and osmo-response might not be taken on the MAPK level or through MAPK regulation of transcriptional activity in the HOG/FG/PH signalling network. Also, the relationship between pheromone and filamentous response cannot be fully accounted for by this approach. Instead, it might be required to take into account cell-cycle regulation, feedback from the cell cycle to the HOG/FG/PH signalling network, and even stochastic effects caused by intrinsic and extrinsic sources of noise within the network [26] to achieve predictivity on this level. Our framework provides possible focal points for future experiments and modelling endeavours to bridge the ‘understanding gap’ between the current consensus view of signalling pathways and the observed emergent phenomena that these networks are thought to cause.

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References

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SUPPLEMENTARY ONLINE DATA

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Equations

\[
\begin{align*}
\frac{d}{dt}[Sln] &= k1[Salt] \frac{k2^n}{k2^n + f1[Hog1]^n} - 13[Sln] \\
\frac{d}{dt}[Sho] &= k1[Salt] \frac{k2^n}{k2^n + f1[Hog1]^n} - k3[Sln] \\
\frac{d}{dt}[Hog1] &= \frac{k4[Sln] + k4[Sho] + k5[Free Ste11]}{1 + \frac{[Kss1]}{k6}} - k3[Hog1] \\
\frac{d}{dt}[Free Ste11] &= k7 \frac{[Nut Dep]}{k8 + [Nut Dep]} + k9[Sho] + k10[Pher] \frac{k2^n}{k2^n + f2[Fus3]} - k7[Free Ste11] \\
\frac{d}{dt}[Kss1] &= k7[Free Ste11] - k7[Kss1] \\
\frac{d}{dt}[Tec1] &= k8 \frac{[Kss1]}{1 + \frac{[Hog1]}{k11} + \frac{[Fus3]}{k12}} - k8[Tec1] \\
\frac{d}{dt}[Ste5 Ste11] &= k13[Pher] \frac{k2^n}{k2^n + f2[Fus3]^n} - k3[Ste5 Ste11] \\
\frac{d}{dt}[Ste12] &= k17[Fus3] - k18[Ste12]
\end{align*}
\]

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Parameter values

\[
\begin{align*}
n &= 2 \\
f_1 &= 1 \\
f_2 &= 1 \\
k_1 &= 5 \\
k_2 &= 0.001 \\
k_3 &= 0.1 \\
k_4 &= 0.2 \\
k_5 &= 0.05 \\
k_6 &= 0.1 \\
k_7 &= 0.2 \\
k_8 &= 1 \\
k_9 &= 0.01 \\
k_{10} &= 3 \\
k_{11} &= 0.1 \\
k_{12} &= 0.1 \\
k_{13} &= 2.5 \\
k_{14} &= 0.4 \\
k_{15} &= 0.005 \\
k_{16} &= 0.1 \\
k_{17} &= 1 \\
k_{18} &= 1
\end{align*}
\]

Pathway stimulation

Pathways are stimulated by adjusting the following parameters:

- \([\text{Salt}] = 10\): osmo-stress on. Otherwise \([\text{Salt}] = 0\).
- \([\text{Nut Dep}] = 10\): nutrient-depletion on. Otherwise \([\text{Nut Dep}] = 0\).
- \([\text{Pher}] = 10\): pheromones on. Otherwise \([\text{Pher}] = 0\).

Software

All simulations were carried out using the Mathworks MATLAB ode45 solver.