FEBS *Letters*

Review

Control of high osmolarity signalling in the yeast *Saccharomyces cerevisiae*

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ABSTRACT

Signal transduction pathways control cellular responses to extrinsic and intrinsic signals. The yeast HOG (High Osmolarity Glycerol) response pathway mediates cellular adaptation to hyperosmotic stress. Pathway architecture as well as the flow of signal have been studied to a very high degree of detail. Recently, the yeast HOG pathway has become a popular model to analyse systems level properties of signal transduction. Those studies addressed, using experimentation and modelling, the role of basal signalling, robustness against perturbation, as well as adaptation and feedback control. These recent findings provide exciting insight into the higher control levels of signalling through this MAPK system of potential general importance.
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1. The yeast HOG pathway

The yeast HOG (High Osmolarity Glycerol) pathway has recently become a popular study object for Systems Biology approaches. The HOG pathway is a branched MAPK (Mitogen Activated Protein Kinase) signal transduction system (Fig. 1). The MAP kinase Hog1 is the yeast orthologue of mammalian p38. The physiological role of the HOG pathway is to orchestrate the adaptation of yeast cells to increased osmolarity of the surrounding medium [1,2]. Such increased medium osmolarity leads to water loss and cell shrinking. The cell needs to counteract those effects in order to maintain shape and turgor and to ensure appropriate water and ion concentration in the cytosol and its organelles for optimal functioning of biochemical reactions. In addition, it has been shown that Hog1 is also required for adaptation to other stress conditions, such as oxidative stress [3], arsenite [4], cold stress [5,6] and acetic acid [7] stress. Orthologues of Hog1 appear to be involved in osmoadaptation and other types of stress responses in probably all eukaryotes, although the specific sensing and regulatory mechanisms as well as the molecular targets of those pathways certainly differ between organisms.

The phosphorylation and hence activity of the Hog1 MAPK is controlled by two branches, the Sln1 and the Sho1 branch, which converge on the MAP kinase kinase (MAPKK) Pbs2. At present, it is not entirely clear why Hog1 is controlled by two branches, because (1) either branch alone can activate Hog1 in response to hyperosmotic stress [8], (2) the Sln1 branch appears to have a far more prominent role in pathway control as it is more sensitive to osmotic changes and supports full pathway activation even in the absence of the Sho1 branch ([9] and our unpublished data), and (3) the Sho1 branch does not seem to be connected to the Hog1 MAPK cascade in a number of other fungi [10]. As discussed further below, it is possible that the Sho1 branch has a role in coordinating signalling between the HOG and other MAPK pathways.

The Sln1 branch is controlled by the plasma membrane-localised sensor Sln1 [11], which spans the membrane twice. Sln1 is related to the two-component osmosensor EnvZ in bacteria and might sense changes in membrane tension and/or turgor [12], although the physical mechanism of osmosensing is still unknown. Sln1, Ypd1 and Ssk1 form a phosphorelay system, the eukaryotic version of the two-component system [11]. Sln1 is active under ambient conditions and inactivated upon hyperosmotic shock. Active Sln1 is a dimer that performs auto-phosphorylation on a histidine. This phospho group is then transferred to a receiver domain in Sln1, further to Ypd1 and eventually to the receiver domain in Ssk1. Phospho-Ssk1 is the inactive form and hence does not activate the downstream MAP kinase cascade [13]. It appears that phospho-Ssk1 is intrinsically unstable or dephosphorylated by an unknown phosphatase. Upon hyperosmotic shock, the level of unphosphorylated Ssk1 rapidly increases. Ssk1 binds to the regulatory domain of the Ssk2 and Ssk22 MAPKKKs [13], which allows Ssk2 and Ssk22 to autophosphorylate and activate themselves. Active Ssk2 and Ssk22 then phosphorylate and activate Pbs2, which in turn phosphorylates (on Thr174 and Tyr176) and activates Hog1 [1,2,8,11,13,14].

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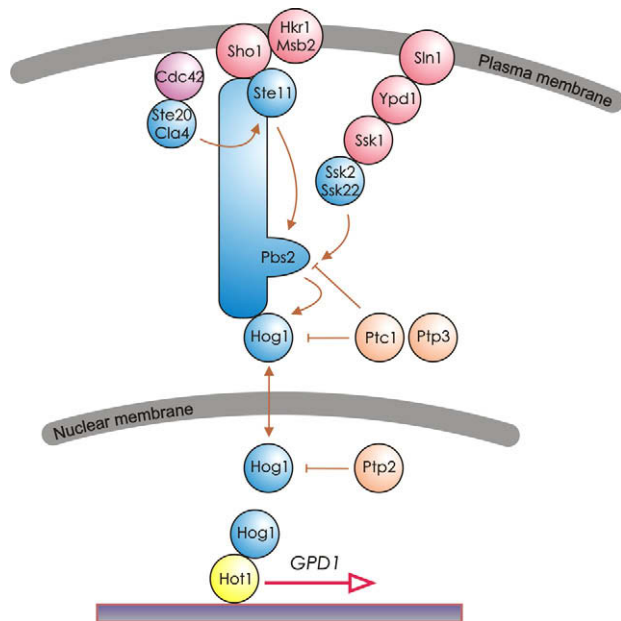*E-mail address:* stefan.hohmann@gu.se

Fig. 1. Components and flow of information in the yeast HOG pathway. Membrane-localised sensors and regulators are shown in red, protein kinases in blue, protein phosphatases in orange and transcription factors in yellow. Two branches converge at the level of Pbs2 to activate Hog1, which accumulates in the nucleus under stress. Ste11, Ssk2 and Ssk22 are MAPKKKs, Pbs2 a MAPKK and Hog1 the MAPK in the system. See text for further details.

The Sho1 branch is controlled by two mucin-like transmembrane sensors, Msb2 and Hkr1 [15,16]. Mucins connect the cell interior with the extracellular matrix (in fungi, the cell wall) and hence may monitor movements between the cell wall and the plasma membrane. Sho1 is an additional transmembrane protein in this branch of the HOG pathway. It has long been believed to be the sensor in the system [8]. However, it appears to play a role as membrane-localised scaffold protein that recruits components to the cell surface at places of active cell surface growth and remodelling [17]. Many molecular details of the activation mechanism remain unknown at this point. It appears to be clear, however, that stimulation of the sensors by hyperosmotic shock results in recruitment to the plasma membrane of Pbs2, which not only serves as MAPKK but also as scaffold for the Sho1 branch [17–20]. Probably, Pbs2 carries along the Ste11 MAPKKK, which thereby is brought into vicinity of the Ste20 and Cla4 kinases. Those are located at the plasma membrane in association with the Cdc42 G-protein. Phosphorylation of Ste11 by Ste20 and/or Cla4 activates Ste11, which then phosphorylates Pbs2, which phosphorylates and activates Hog1 [1,2,21].

The phosphorylation state of the MAPK Hog1 is controlled by various protein phosphatases. Those include the phospho-tyrosine phosphatases Ptp2 and Ptp3 [22–24] as well as the phospho-threonine phosphatase Ptc1 (Ptc2 and Ptc3 also seem to play a role, at least when over-expressed) [14,25–27].

Acute hyperosmotic stress leads to a rapid (sub-minute) increase in the amount of phospho-Hog1, the active form of the kinase. Under such conditions, phosphorylation is accompanied (and required for) import of Hog1 into the nucleus [28], where the kinase participates on target promoters in the control of gene expression [29,30]. However, the kinase also has targets in the cytosol [4,31,32], which suggests that not all Hog1 is recruited to the nucleus. In fact, by tethering Hog1 to the plasma membrane and in this way preventing its nuclear import, it has been demonstrated that the activation of gene expression is NOT critical for osmoadaptation [33]. The targets in the cytosol that confer the

essential steps in osmoadaptation mediated by Hog1 remain to be identified.

Hog1 is one of four MAPKs in *Saccharomyces cerevisiae* [34]. The other three are: (1) Fus3, which controls the response to mating pheromones, (2) Kss1, which controls morphogenic switches in response to nutrient conditions and (3) Slt2/Mpk1, which controls cell surface remodelling following hypo-osmotic shock and in response to morphogenic signals such as pheromone treatment. The common theme for all yeast MAPKs is, hence, the control of cell growth and morphogenesis in response to different environmental stimuli. Probably all MAPKs also affect cell cycle control. In the case of Hog1 [35–39] and Fus3 [40,41] the underlying molecular details are well documented. In this way, MAPKs coordinate the control of cell morphogenesis with that of the cell division cycle. As can be expected from the joint role in controlling cell morphogenesis, the four yeast MAPK pathways communicate at various levels. In fact, the Fus3 and Kss1 pathways as well as the Sho1 branch of the HOG pathway share protein kinases and all MAPK pathways share protein phosphatases. In several instances, the points of pathway interaction have been identified and documented at the molecular level, e.g. [42,43], but much work remains to map the yeast MAPK network and the flow of information in detail. Given that, in spite of its complexity, the yeast MAPK system is simple when compared to that of plants and animals, it provides significant opportunities for molecular and system-level studies in the near future.

2. HOG controls glycerol accumulation in osmoadaptation

As the name states, the probably most important role of the HOG pathway in osmotic adaptation concerns the control of glycerol accumulation. Glycerol serves as the osmolyte of proliferating yeast cells [1]. It performs this role by partly replacing water and protecting biomolecules inside the cell as well as by increasing the intracellular water potential and thereby driving back water into the cell. The strategy of accumulating osmolytes is conserved in all kingdoms of life; the nature of the osmolyte however differs between genera and species.

Glycerol is produced by yeast cells for two reasons: osmoregulation and redox-balancing. It is hence a common by-product of yeast fermentations. Glycerol is produced from the intermediate of glycolysis, dihydroxyacetonephosphate, into two steps. Those are catalysed by glycerol-3-phosphate dehydrogenase (Gpd1 and Gpd2 in *S. cerevisiae*) and glycerol-3-phosphatase (Gpp1 and Gpp2) [1].

Hog1 appears to control glycerol accumulation at several steps (Fig. 2). Those include (1) the expression of the genes encoding Gpd1, Gpp1 and Gpp2, which is up-regulated in a partly HOG-dependent manner after hyperosmotic shock [44,45]; (2) the expression of the Stl1 active glycerol uptake system [44,45], which allows accumulating glycerol from the surrounding medium [46]; (3) the activity of the enzyme phosphofructo-2-kinase, which produces the glycolytic activator fructose-2,6-bisphosphate – this effect appears to increase the rate of glycerol production ([47] and our unpublished data); and (4) control of the activity of the glycerol export channel Fps1 [4].

Fps1 clearly plays a critical role in controlling the intracellular level of glycerol, and mutations which render Fps1 unregulatable cause glycerol leakage and diminished ability of yeast cells to adapt to hyperosmotic stress [48,49]. The mechanism by which Hog1 controls Fps1 (gating, stability, trafficking) remains unclear at the moment. Remarkably, it appears that the role of Hog1 in the acquisition of tolerance to arsenite [4] and acetic acid [7] is confined to controlling Fps1, which is an entry pathway for those substances. Aquaglyceroporins, such as Fps1, are known to
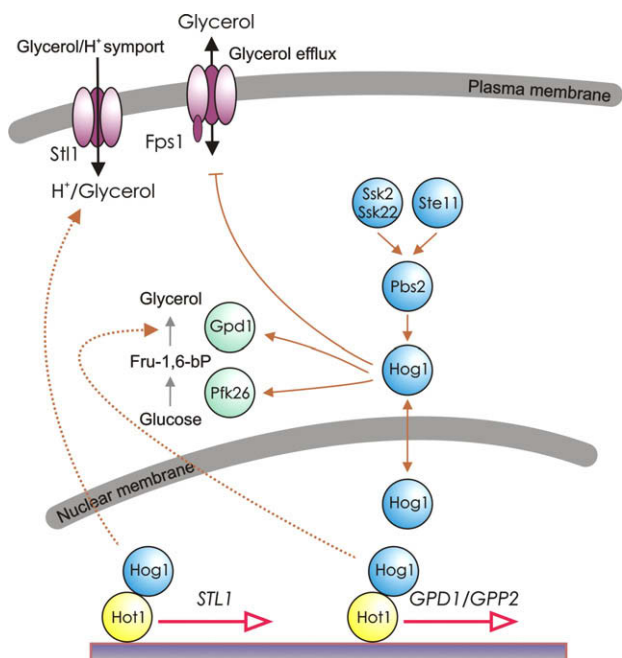
Fig. 2. Mechanisms by which Hog1 controls glycerol accumulation. The HOG pathway is only represented schematically. Protein kinases are indicated in blue, transcription factors in yellow, enzymes in green and transmembrane transporters in violet. Broken lines represent protein production rather than regulation. See text for further details.

mediate passive transport of compounds that resemble glycerol structurally.

The yeast HOG pathway is without doubt one of the best understood MAPK signalling systems. Genetics and molecular studies have revealed the components, the interaction map and the flow of the signal in the system. Because of this high degree of molecular understanding, the ease of perturbing the system in a well-defined manner by osmotic shock treatments and the possibility of generating quantitative data at cell population as well as single-cell level has made the HOG network a favourite for systems level analyses. Recent work from various laboratories has shed new light on the mechanisms that control signalling through the HOG pathway.

3. Control of basal signalling in HOG signalling

Hog1 appears to be unphosphorylated under ambient conditions while it becomes rapidly phosphorylated following hyperosmotic shock. Constitutive Hog1 phosphorylation, as achieved by various mutations, is lethal. This lethality is probably due to the inhibitory effect of active Hog1 on cell cycle progression. One such mutation that causes lethality due to constitutive Hog1 activation is *ptc1Δ ptp2Δ*, eliminating two protein phosphatases [27]. This has been known since the genetic screens leading to the identification of the first HOG pathway components. The observation suggests that there is an intrinsic HOG signalling activity even in the absence of stress, which is permanently down-regulated by protein phosphatases at the level of Hog1. Interestingly, this down-regulation appears to depend on Hog1 activity: a kinase-dead (activity eliminated) mutant version of Hog1 is constantly phosphorylated even in the absence of stress. Moreover, a Hog1-as mutant, which has been made sensitive by mutation to bulky ATP-analogues, becomes phosphorylated upon inhibition within a couple of minutes. In conclusion, there is intrinsic HOG signalling activity, which is counteracted by a mechanism that requires Hog1 activity [50].

Theoretical considerations lead the groups of Solé and Posas to conclude that such basic signalling may serve a specific purpose [50]. It may allow more rapid response and also may allow fine-tuning of signalling thresholds upon stress treatments. The team identified that the source for basal signalling is solely the Sln1 branch of the network. What remains to be determined, however, are (1) the point(s) within the pathway where basal signalling is generated as well as (2) the mechanisms by which basal signalling is controlled, i.e. the target of Hog1 activity in this context. While the most apparent candidates are protein phosphatases no evidence supporting this idea has been produced so far. Control by Hog1 of an upstream component of the pathway remains a possibility.

4. Adaptation and feedback control of HOG signalling

Hog1 becomes phosphorylated rapidly after hyperosmotic shock but the level of phosphorylated Hog1 drops to about pre-stress levels within a period of time that depends on the degree of stress. This transient nature of the response is visible not only at the level of Hog1 phosphorylation [51] but is also reflected by transient nuclear localisation of Hog1 [28], transient appearance of Hog1 on target promoters [52] and transient stimulation of gene expression [45]. Hence, activation of Hog1 is controlled by feedback regulation.

Several possibilities exist how Hog1 might down-regulate its own activation. For instance, upstream components such as Ste11 or Sho1 [53] might be controlled by Hog1. It also has been reported that Ssk1 stability [54] depends on its phosphorylation state. A popular, and still often cited scenario, concerns the transcriptional stimulation of the genes *PTP2* and *PTP3*, which could result in an increased specific phosphatase activity to down-regulate Hog1 phosphorylation [24]. However, mathematical modelling and simulation of the feedback control system demonstrated that the rather modest transcriptional stimulation (alone) can not explain the rigorous down-regulation to pre-stress levels, especially not under situations where pathway activation only last a few minutes [51].

Instead it appears that osmotic adaptation and thereby signal cessation controls the period of HOG pathway activation. First of all, such a scenario could best explain existing experimental data when simulated using a mathematical model [51]. In addition, there are several experimental observations that support a scenario where cellular adaptation controls the period of Hog1 activation: (1) as mentioned above, the period of Hog1 phosphorylation depends on the degree of stress (longer at higher concentrations of osmoticum), where adaptation can be expected to take longer [51]; (2) mutants with a decreased ability to accumulate glycerol show a longer period of Hog1 phosphorylation [51]; (3) mutants with an enhanced ability to accumulate glycerol show a shorter period of Hog1 phosphorylation [55]; (4) in a semi-artificial adaptation scenario, the period of Hog1 phosphorylation appears to correlate with the time it takes for cells to adapt [56]; and (5) when shifted back from hyperosmotic conditions to ambient conditions Hog1 becomes dephosphorylated extremely rapidly ([12] and own unpublished data). Taken together, it appears that Hog1 is constantly phosphorylated at a high rate under hyperosmotic stress but as soon cells adapt to the conditions (i.e. re-swell again) the phosphorylation drops such that protein phosphatases cause dephosphorylation of Hog1. In this scenario, supported by modelling and simulation, other Hog1-driven feedback control mechanisms are not needed to reproduce the data, but are well possible to exist.

Van Oudenaarden's laboratory followed up on these observation employing single-cell analysis and Hog1 nuclear shuttling as

a readout, combined with straightforward mathematical modelling [57,58]. The work made use of microfluidic devices that allow rapid and precise changes in external osmolarity and microscopic observation and quantification of Hog1 nuclear-cytosolic shuttling. This work confirmed that the Hog1 system has the ability to perfectly adapt. Moreover, it was observed that the dynamics of the osmoadaptation response is characterised by a fast-acting negative feedback through Hog1 that does not require protein synthesis. At stronger stress levels an additional slower negative feedback, which appears to require gene expression, allows cells to respond faster to future stimuli [58].

Searching for the nature of the mechanisms that allow perfect adaptation the group continued to monitor single-cell dynamics. They found that the nuclear enrichment of the MAP kinase Hog1 perfectly adapts to changes in external osmolarity, a feature that is robust to signalling fidelity and operating with very low noise. By monitoring multiple system parameters such as cell volume, Hog1 localisation and glycerol levels after various input waveforms the location of the mechanism responsible for perfect adaptation was narrowed down [57]. Not unexpectedly, the mechanism requires Hog1 kinase activity. It appears to control glycerol production but, somewhat unexpectedly, not leakage through Fps1. Taken together, these observations confirm that both activation and deactivation of HOG signalling are controlled by osmotic rather than pathway-intrinsic events. Our data, however, indicate that different, perhaps all of the HOG-dependent events in glycerol accumulation contribute to adaptation to various degrees (our unpublished data). While we agree that many of the observations can be explained by much simpler models than that presented by Klipp et al. [51], it is important to consider the complexity of cellular response mechanisms, which commonly are fine-tuned at various levels.

5. Other pathway control mechanisms

In a recent study the robustness of the HOG pathway to strong increases in the level of its components was monitored [59]. Over-expression of several components of the Sln1 branch resulted in lethality because of constitutive, unregulated Hog1 activation. As was known previously, over-expression of Pbs2 causes such an effect. In addition, also over-expression of Ssk1 caused lethality. In each case, the lethality caused by over-expression could be suppressed by deletion of genes encoding components downstream in the pathway, consistent with the idea that pathway over-activation causes lethality. Interestingly, however, the lethality caused by over-expression of Pbs2 was also suppressed by deletion of *SSK2*, encoding the kinase upstream of Pbs2. These observations can be seen in light with those on basal signalling in the Sln1 branch of the pathway [50]. It appears that over-expression of Pbs2 amplifies a low constitutive signal that originates from an upstream component, perhaps Ssk1, and which is normally counteracted by protein phosphatases acting on Pbs2 and/or Hog1. The effect caused by over-expression of Ssk1 may be explained by the fact that it is the unphosphorylated form of Ssk1 that is active towards Ssk2/Ssk22. Over-expression may overrule the capacity of the Sln1–Ypd1 pair to phosphorylate and down-regulate Ssk1. Interestingly, over-expression of Ssk2 nor Ssk22 only caused moderate growth inhibition. Perhaps this is due to the fact that Ssk2/Ssk22 needs to interact with dephospho-Ssk1 in a stoicheometric fashion for activation [59].

6. Systems-level properties of HOG signalling

In conclusion, the yeast osmoregulating HOG pathway is a suitable model to study systems level properties of signalling path-

ways. Although open questions concerning molecular details remain, the pathway appears to be understood to a very high degree. Recent observations elucidate specific properties concerning adaptation, feedback control, robustness to fluctuations in component number or activity as well as a role of basal signalling in controlling thresholds and noise. Numerous interesting questions remain to be studied. Those include, for instance, the quantitative and temporal contributions to glycerol accumulation of different HOG-dependent control mechanisms or the mechanisms that control the cross-talk between the HOG and other yeast MAPK pathways in orchestrating cellular morphogenesis.

Acknowledgements

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