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Minireview

The HOG pathway and the regulation of osmoadaptive responses in yeast

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One sentence summary: The authors discuss the high-osmolarity glycerol pathway and stress adaptation in yeast.
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Abstract

Cells coordinate intracellular activities in response to changes in the extracellular environment to maximize their probability of survival and proliferation. Eukaryotic cells need to adapt to constant changes in the osmolarity of their environment. In yeast, the high-osmolarity glycerol (HOG) pathway is responsible for the response to high osmolarity. Activation of the Hog1 stress-activated protein kinase (SAPK) induces a complex program required for cellular adaptation that includes temporary arrest of cell cycle progression, adjustment of transcription and translation patterns, and the regulation of metabolism, including the synthesis and retention of the compatible osmolyte glycerol. Hog1 is a member of the family of p38 SAPKs, which are present across eukaryotes. Many of the properties of the HOG pathway and downstream-regulated proteins are conserved from yeast to mammals. This review addresses the global view of this signaling pathway in yeast, as well as the contribution of Dr Hohmann's group to its understanding.

Keywords: osmostress, HOG pathway, stress adaptation

Introduction

Yeast cells have to adapt to constant shifts in their environment. This adaptation requires major coordinated changes in cell physiology to ensure cell adaptation and survival. Mitogen-activated protein kinase (MAPK) signaling cascades are highly conserved across eukaryotes. These pathways, which sense a wide variety of stimuli and respond to extracellular cues through sequential activation of protein kinases, are involved in a myriad of fundamental cellular processes and determine cell fate. The misregulation of these signaling cascades, therefore, has major consequences in numerous diseases, including cancer, diabetes, and inflammatory and immune response diseases. The Hog1/p38 family of MAPKs includes stress-activated protein kinases (SAPKs) activated in response to several stresses in a variety of organisms. The response to variations in extracellular osmolarity has been evolutionarily conserved, and it involves the activation of the p38/Hog1 family of signaling cascades. In *Saccharomyces cerevisiae*, osmostress induces the high-osmolarity glycerol (HOG) pathway, which includes Hog1. Upon activation in response to osmostress by upstream sensors and effectors, Hog1 induces a cytoplasmic response that acts on glycerol and ion transporters, metabolism and translation. Additionally, Hog1 rapidly translocates into the nucleus, where it modulates transcription to regulate gene expression and alters cell cycle progression.

Osmostress-adaptive responses

In response to changes in the extracellular environment, cells coordinate several intracellular activities to maximize their probability of survival and proliferation. Many of the stress responses required for adaptation, which range from the regulation of metabolism, transcription and translation to cell cycle progression, are modulated by the HOG pathway (Fig. 1).

Exposure to increased osmolarity is known to result in loss of water, cell shrinkage and a temporary arrest of growth until adaptation occurs. A major survival strategy under high osmolarity is to produce and accumulate compatible osmolytes, such as amino acids, ions, trehalose and, more importantly, glycerol, to maintain the water balance and re-establish cell volume and turgor (Blomberg and Adler 1989, Hohmann *et al.* 2007, Westfall *et al.* 2008, de Nadal *et al.* 2011). The initial function of the HOG pathway in stress adaptation was rapidly associated with the regulation of metabolism and the production of osmolytes to counteract the loss of water upon osmostress. Mutants in the HOG pathway yielded cells unable to grow under high osmolarity and this was rapidly associated with the lack of proper adaptation (Saito and Posas 2012). Dr Hohmann's group, who was studying how metabolic changes and certain metabolites, such as trehalose and glycerol, counteracted high osmolarity, rapidly realized that the HOG pathway regulated the transcription of key enzymes required for glycerol production and stress adaption (Hounsa *et al.* 1998, Rep *et al.* 1999a). Later on, evidence emerged of direct and allosteric regulation of metabolic enzymes (Dihazi *et al.* 2004, Hohmann *et al.* 2007). In this regard, the carbon source determines adaptation to osmostress (Babazadeh *et al.* 2017). Glycerol is rapidly accumulated in response to osmostress, starting within the first minute and with significant accumulation after 30 min (Klipp *et al.* 2005, Stojanovski *et al.* 2017). Therefore, an increase

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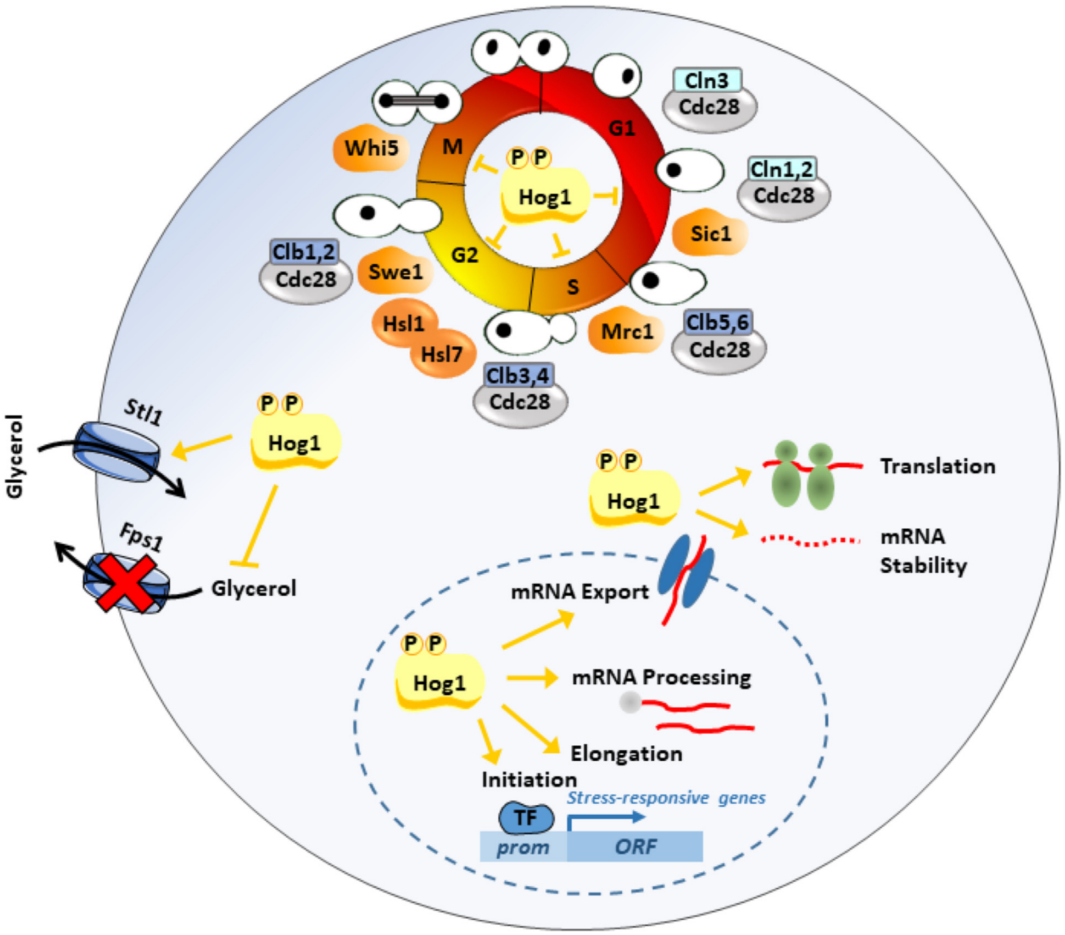

Figure 1. Upon osmostress, activated Hog1 orchestrates several cellular functions to coordinate the adaptive response and maximize cell survival. Once activated, Hog1 controls mRNA biogenesis in both the nucleus and the cytoplasm. In the nucleus, Hog1 associates with stress-responsive loci to modulate transcription initiation and elongation. The cyclin-dependent kinase Cdc28 associates with phase-specific cyclins (shown around the central circle) to regulate passage through the cell cycle. Upon stress, Hog1 modulates progression at all phases of the cell cycle by acting on the indicated core elements of the cell cycle machinery. Hog1 also induces a cytoplasmic response that acts on glycerol and ion transporters, metabolism and translation.

in glycerol production is not the only mechanism by which cells accumulate this metabolite as they also regulate glycerol export and import (Hohmann 2002, Klipp *et al.* 2005, Saito and Posas 2012). Stl1, a sugar transporter-like protein whose expression is strongly induced by Hog1 upon stress, might contribute to glycerol accumulation by importing it from the environment in response to stress. However, the fastest mechanism to alter glycerol concentration is via Fps1-mediated export of this metabolite (Tamás *et al.* 1999). Fps1 is a member of the aquaporin family of transmembrane channels—a family of proteins critical for stress responses that has been extensively studied by Dr Hohmann's group (e.g. Karlgren *et al.* 2005; review in this issue). Cells that express Fps1 mutant proteins that are constitutively open do not accumulate glycerol and grow poorly in the presence of high osmolarity (Hohmann *et al.* 2007). The stress-induced phosphorylation of Rgc2, a novel regulator of Fps1 channel activity, is also partially controlled by Hog1 to regulate glycerol efflux (Mollapour and Piper 2007, Beese *et al.* 2009, Lee *et al.* 2013). The role of the SAPK pathway in osmoadaptation, including its impact on metabolism, was reviewed early on by Hohmann (2002).

The regulation of gene expression is a key hallmark of adaptive responses to stress (de Nadal *et al.* 2011, de Nadal and Posas 2015). Initially and as mentioned earlier, it was clear that the expression of several genes involved in the regulation of metabolism, including trehalose, glycogen and glycerol synthesis, is upregulated upon stress, in parallel with sugar transporters. This observation prompted interest in the role of the HOG pathway and its downstream transcription factors in this regulation. Stress causes a general downregulation of gene expression, combined with the induction of a specific set of stress-responsive genes (environmental stress response (ESR) response), resulting in a change in the gene expression landscape of the cell. Expression profiling studies have shown that ~300–600 genes are regulated upon stress (Rep *et al.* 1999b, 2000, Gasch *et al.* 2000, Posas *et al.* 2000, Causton *et al.* 2001, O'Rourke and Herskowitz 2004, Tomas-Cobos *et al.* 2004, Capaldi *et al.* 2008, Molin *et al.* 2009, Ni *et al.* 2009, Romero-Santacreu *et al.* 2009, Miller *et al.* 2011, Nadal-Ribelles *et al.* 2012) and have revealed a broader role of Hog1 as a master regulator of the massive transcriptional reprogramming that occurs upon stress (de Nadal and Posas 2015). Of note, the induction of stress-responsive genes is strongly linked to volume regulation (Geijer *et al.* 2013) and it depends on the state of chromatin (Pelet *et al.* 2011, Wosika and Pelet 2020). Hog1 is a master protein for reprogramming gene expression in response to osmostress through various transcription factors (Rep *et al.* 2000, Capaldi *et al.* 2008, Ni *et al.* 2009, Nadal-Ribelles *et al.* 2012). Hog1 is recruited to osmoresponsive genes by these specific factors (Alepuz *et al.* 2001, 2003, Proft *et al.* 2001, 2006, Proft and Struhl 2002, de Nadal *et al.* 2003,

Pascual-Ahuir *et al.* 2006, Pokholok *et al.* 2006, Ruiz-Roig *et al.* 2012). Once bound to chromatin, Hog1 serves as a platform to recruit RNA polymerase II (Alepuz *et al.* 2003, Nadal-Ribelles *et al.* 2012) and associated factors such as SAGA, Mediator and the histone deacetylase Rpd3 (Proft and Struhl 2002, de Nadal *et al.* 2004, Zapater *et al.* 2007, Sole *et al.* 2011). Hog1 is also present at the coding regions of stress-responsive genes (Pascual-Ahuir *et al.* 2006, Pokholok *et al.* 2006, Proft *et al.* 2006, Nadal-Ribelles *et al.* 2012), as well as at lncRNAs (Nadal-Ribelles *et al.* 2014), where its kinase activity is essential for increased association of RNA polymerase II and efficient messenger RNA (mRNA) production in response to osmostress (Proft *et al.* 2006, de Nadal and Posas 2011, Cook and O'Shea 2012, Nadal-Ribelles *et al.* 2012). Moreover, nucleosome positioning of specific stress-responsive loci is altered dramatically in a Hog1-dependent manner (Nadal-Ribelles *et al.* 2012) and the modification of chromatin is regulated mostly by the interplay of the INO80 and the RSC chromatin remodeling complexes (Klopf *et al.* 2009, Mas *et al.* 2009, Nadal-Ribelles *et al.* 2015). Chromatin dynamics set a threshold for gene induction upon Hog1 activation (Pelet *et al.* 2011). In addition to its impact on gene induction, Hog1 governs mRNA stability (Molin *et al.* 2009, Romero-Santacreu *et al.* 2009, Miller *et al.* 2011), mRNA export by targeting specific nucleoporins in the nuclear pore complex (Regot *et al.* 2013) and mRNA translation (Teige *et al.* 2001, Warringer *et al.* 2010). Thus, this SAPK plays a key role in the regulation of mRNA biogenesis by controlling several steps in the transcriptional process (Hohmann 2002, de Nadal and Posas 2010, Martinez-Montanes *et al.* 2010, de Nadal *et al.* 2011).

Another critical function required for stress adaptation is cell cycle regulation. The HOG pathway was initially deciphered through genetics, thanks to the observation that mutations that resulted in the sustained activation of this pathway were deleterious for cellular growth (Maeda *et al.* 1993, 1994). Later on, it was shown that sustained activation of HOG led to cell cycle arrest, which, if maintained, resulted in cell entry into apoptosis (Vendrell *et al.* 2011). In contrast, the natural transient activation of the HOG pathway in response to osmostress causes a transient cell cycle delay that is essential for cell survival upon stress. Thus, cells activate checkpoint surveillance mechanisms to permit adaptation to environmental conditions. Hog1 regulates multiple stages of the cell cycle by acting on core components of the cell cycle machinery. For instance, Hog1 controls G1/S transition by downregulating cyclin expression and stabilizing the Sic1 cyclin-dependent kinase inhibitor (Escote *et al.* 2004, Adrover *et al.* 2011, Gonzalez-Novo *et al.* 2015). Hog1 also regulates other phases of the cell cycle, such as S phase (Yaakov *et al.* 2009, Duch *et al.* 2013, 2018), G2/M phase (Alexander *et al.* 2001, Clotet *et al.* 2006) and mitosis in response to stress (Jiménez *et al.* 2020, Tognetti *et al.* 2020). These observations thus suggest that, in the presence of stress, a delay in the cell cycle is required to allow cells to generate adaptive responses before progressing into the next phase of the cycle.

The hog pathway and its activation dynamics upon stress

The HOG pathway is activated in response to osmostress as a result of signaling elicited from two upstream-independent mechanisms (Sln1/Sho1) (Fig. 2). The Sln1 sensor is the primary osmosensor and it is a complex variation of the well-known bacterial two-component system. Upon osmostress, inactivation of the transmembrane histidine kinase Sln1 leads to the derepression and activation of MAP3Ks (Ssk2/22) via Ypd1/Ssk1 (Maeda *et al.* 1995, Posas *et al.* 1996, Posas and Saito 1998). The osmosensors of the Sho1 branch are the mucin-like proteins Msb2 and Hkr1, which are transmembrane proteins with a highly glycosylated extracellular domain (Tatebayashi *et al.* 2007). Through complex interactions with different proteins (Saito and Posas 2012), these two osmosensors, along with Sho1, are responsible, in collaboration with the integral membrane protein Opy2, for the activation of the small rho-like GTPase Cdc42 and subsequent activation of the kinases Ste20 and Cla4, which in turn lead to activation of the Ste11 MAP3K (Drogen *et al.* 2000, Raitt *et al.* 2000, Lamson *et al.* 2002). The Sln1 and Sho1 branches converge at the Pbs2 MAP2K (Brewster *et al.* 1993, Maeda *et al.* 1995, Posas and Saito 1997), which is activated by phosphorylation albeit with different modes of activation (Tatebayashi *et al.* 2020). Once Pbs2 is active, it can phosphorylate Hog1 and this phosphorylation is accompanied by an immediate translocation of Hog1 to the nucleus (Ferrigno *et al.* 1998), where it performs many of the adaptive responses of yeast cells to osmostress. The activation dynamics of Hog1 and crosstalk among different pathways are critical to understand stress adaptation and to the contribution of distinct regulatory elements to stress-adaptive responses. The activation of Hog1 is mediated by phosphorylation and it occurs within seconds of exposure to stress. The amplitude and duration of this activation depend on the strength of the stress and it is governed by not only the upstream sensing mechanisms but also the negative regulators of the pathway (e.g. protein phosphatases) and internal feedback loops that counteract activating signals and integrate, for instance, membrane turgor and glycerol levels. The relevance of quantitatively assessing the signaling of the pathway and the contribution of the individual regulatory elements by mathematical modeling was clearly exemplified by a seminal paper by the groups led by Drs Hohmann and Klipp demonstrating the role of osmolyte accumulation and feedback control in HOG dynamics (Klipp *et al.* 2005, Petelenz-Kurdziel *et al.* 2013). Population and single-cell studies using modeling and systematic quantification of signaling under different types of perturbation (environmental and genetic) have permitted the research community to determine weaknesses in the pathway topology and the role of scaffolding in signaling properties (Krantz *et al.* 2009). These approaches have also served to reveal that the HOG pathway is not an ON and OFF signaling system that is activated only when stress occurs but rather a system that is constantly ON and counteracted by internal negative feedback. Upon osmostress, an increase in signaling results in faster induction of Hog1, which would be achieved by activation from a non-active state (Macia *et al.* 2009, Muzzey *et al.* 2009, Petelenz-Kurdziel *et al.* 2011, Babazadeh *et al.* 2013). Thus, the control of the HOG pathway dynamics is governed by not only phosphatases, highlighting the significance of constantly counteract pathway activation and osmolyte production to adjust the dynamic range and the activation threshold of the response rather than the deactivation of the pathway during adaptation (Klipp *et al.* 2005, Hohmann 2009, Schaber *et al.* 2010, Johnson *et al.* 2021), but also several negative feedback regulatory loops (Hao *et al.* 2007, 2008, Mettetal *et al.* 2008, Macia *et al.* 2009, Muzzey *et al.* 2009, Sharifian *et al.* 2015). Thus, it is not surprising that the knowledge gathered about this signaling pathway, in terms of components, response dynamics and regulatory loops, has been exploited in synthetic biology applications. A clear example of this is the use of the HOG pathway to rewire signaling in biological computation applications (Regot *et al.* 2011). The HOG pathway has also been used to control cell fate regulation in response to a fungicide-responsive heterologous histidine kinase (Meena *et al.* 2010, Furukawa and Hohmann 2015) or in the creation of a bistable toggle switch (Mishra *et al.* 2021).

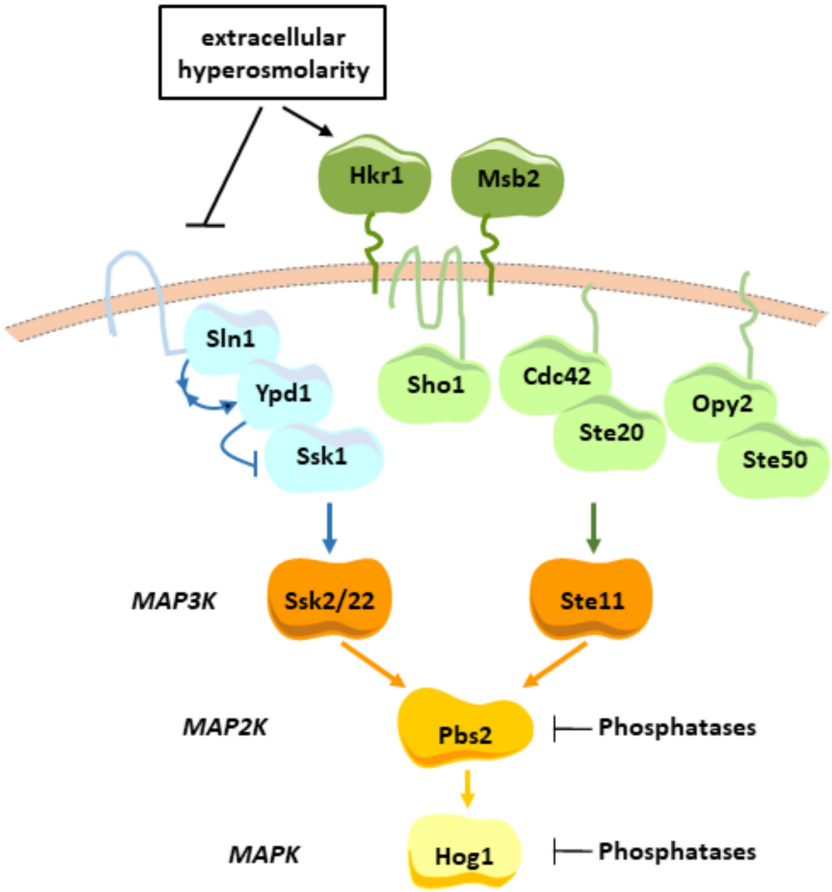

Figure 2. Schematic diagram of the HOG pathway. In response to osmostress, two independent upstream osmosensing mechanisms, the Sln1 and Sho1 branches, lead to the activation of specific MAP3Ks (Ssk2/22 and Ste11) that converge on the common Pbs2 MAP2K. Activated Pbs2 phosphorylates the Hog1 MAPK, which induces a set of adaptive responses.

Conclusions

The HOG pathway plays a pivotal role in osmostress signal transduction and has been extensively studied. Since its discovery in the mid-1990s, it has served as a paradigm of a signal transduction pathway conserved across eukaryotes and has also been used to decipher a number of stress responses required for stress adaptation, which is also highly conserved across eukaryotes. The initial knowledge gathered about the elements of the pathway and their regulation was defined mostly by genetic and biochemical approaches. However, the use of cell biology technologies, microfluidics, single-cell analyses, quantitative biology and mathematical modeling has served to not only deeply characterize stress responses and signal transduction but also use this pathway in synthetic biology applications and pioneer technologies that have been used in many other signal transduction pathways in yeast and other organisms. Dr Hohmann's studies have been a clear example of this evolution in the study of a pathway, from the initial link of the signal transduction to metabolic regulation, to the quantification and modeling of signal transduction and its impact on cell biology, to its manipulation for synthetic biology applications.

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