Dynamic processes at stress promoters regulate the bimodal expression of HOG response genes

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Osmotic stress triggers the activation of the HOG (high osmolality glycerol) pathway in *Saccharomyces cerevisiae*. This signaling cascade culminates in the activation of the MAPK (mitogen-activated protein kinase) Hog1. Quantitative single cell measurements revealed a discrepancy between kinase- and transcriptional activities of Hog1. While kinase activity increases proportionally to stress stimulus, gene expression is inhibited under low stress conditions. Interestingly, a slow stochastic gene activation process is responsible for setting a tunable threshold for gene expression under basal or low stress conditions, which generates a bimodal expression pattern at intermediate stress levels.

Extra- and intracellular stress conditions transiently activate dedicated stress response pathways to allow cells to adapt to the new environment. In *Saccharomyces cerevisiae*, osmotic stress results in the activation of the HOG MAPK pathway, where surface proteins sense changes in the osmolarity of the medium and relay this signal to Ste11 or Ssk2,22. Either one of these MAPK kinase kinases can phosphorylate the MAPK kinase Pbs2, which in turn doubly-phosphorylates and thereby activates the MAPK Hog1. Like all other MAPKs, Hog1 has a dual function: it directly phosphorylates targets to rapidly react to the imposed condition and it induces the synthesis of new proteins to establish a cellular environment adapted to the stimulus.

Through its kinase activity, Hog1 drives the accumulation of glycerol in the cell by modulating the cellular metabolism to increase the production of glycerol and by closing glycerol export channels at the plasma membrane (Fig. 1A). Once the accumulation of glycerol allows the internal and external osmotic pressures to reach a new equilibrium, the cell will resume growth and cell cycle progression.2 Depending on the level of stress imposed on the cell, the amount of glycerol that accumulates varies: low osmo-stresses (0.05 M NaCl) require the production of a small amount of glycerol and are therefore quickly overcome (~5 min), while high osmo-stresses (0.4 M NaCl) require a large increase in internal glycerol concentration and therefore result in a longer activation of the pathway (~30 min.). Thus, the activation of the HOG pathway by a defined amount of osmo-stress opens a transient window of Hog1 activity.

Surprisingly, even relatively high stresses (~0.5 NaCl) can be overcome without the induction of a transcriptional program and de novo protein synthesis.3 Moreover, the transient Hog1 activity, as measured by its nuclear relocation, is unaffected by inhibiting translation with cycloheximide,4 implying that the duration of the temporal window of Hog1 activity largely depends on the adaptation response of the cells. However, in parallel to its cytoplasmic activity driving glycerol accumulation, Hog1 is also active in the nucleus and required to induce a transcriptional program, which affects the expression of roughly 900 genes, including temporal downregulation of growth-associated genes and upregulation of a common set of 300 environmental stress genes.5,6 Although these genes are not

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required for the immediate response to stress, they adjust the cellular metabolism to the new conditions and improve the resistance to various kinds of future insults.

Hog1 has been implicated in many of the steps required for efficient mRNA transcription (Fig. 1A). First, Hog1 binds to a set of transcription factors (TF), which reside on the promoters even in the absence of stress, and thus serve as a specific recruitment platform for the MAPK to stress genes. Subsequently, RNA polymerase II (PolII) is recruited to the promoter by the Hog1-TF complex, and MAPK-dependent chromatin remodeling by the SAGA (Spt/Ada/GCN5/Acetyltransferase) and RSC (Remodeling Structure of Chromatin) complexes allows PolII displacement on the open reading frame. Finally Hog1 also is found in the coding region acting as an elongation factor. Because the kinase activity of Hog1 is required for all these steps, the transcriptional induction of stress genes will stop as soon as adaptation has occurred.

Importantly, when quantitatively studying Hog1-dependent expression at the single cell level using fluorescent expression reporters, we recently discovered a striking discrepancy between Hog1 activity and gene expression. While low stresses (0.05 M NaCl) result in a clear activation of the MAPK and its relocation to the nucleus, they fail to trigger reporter expression. As stress levels gradually increase (0.75–0.15 M NaCl), the response of the population is split in expressing and non-expressing cells, generating a bimodal distribution. At higher stresses (≥0.2 M NaCl) Hog1-driven expression is observed in all cells and increases proportionally to the applied stress (Fig. 2A). The noise in gene expression observed at 0.1 M NaCl can be attributed to intrinsic factors (variations within a given cell), since the expression of two fluorescent reporter variants under the control of the same stress-inducible promoter in single cells is not correlated.

Interestingly, tailored modulation of the dynamics of Hog1 activity allowed us to demonstrate that this bimodal response is caused by the combination of a transient adaptation response and a slow stochastic activation of gene expression. While the adaptation process mainly depends on high copy number molecules (MAPK cascade, glycolytic enzymes, glycerol) and thus occurs surprisingly uniformly across the cell population, the expression of stress-induced proteins is a highly stochastic process since multiple consecutive reactions have to take place on a single locus. Therefore, when the adaptation time is similar to the average time required for the activation of transcription, only a fraction of the cells is able to express the fluorescent stress reporter (Fig. 1B). As predicted by this model, a higher threshold of expression and an amplified bimodal expression behavior are observed in mutants defective for chromatin remodeling (gcen5Δ, rsc9-ts) or deletions of specific transcription factors (hot1Δ, sko1Δ) implying that these mutants require a longer period of Hog1 activity to become transcriptionally active. Conversely, in cells deficient for nucleosome deposition (arp8Δ, a member of the Ino80 complex),

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Figure 1. Schematic representation of Hog1 driven osmo-adaptation and gene expression. (A) Upon osmotic shock, Hog1 is rapidly activated and triggers a series of processes leading to cytoplasmic glycerol accumulation and cellular adaptation, which in turn inactivates Hog1. In parallel, Hog1 induces a transcriptional response by promoting a series of kinase-dependent steps at stress-specific promoters, which are required for efficient protein expression. (B) As the level of osmotic stress increases, the duration of the adaptation process increases, thus allowing more time for Hog1 to catalyze all the steps required for gene expression. This time-dependent mechanism sets an expression threshold and results in a bimodal expression pattern with a larger fraction of cells able to produce the expression reporter as stress levels increase.
expression of stress-specific fluorescent reporters is already detected in a significant fraction of unstimulated cells. As expected, these effects can compensate each other; for example, the strong defect in reporter expression in hot1Δ cells can partially be restored by additional deletion of ARP8. Together, these data imply that any factor modulating the dynamic process of gene activation will influence the bimodal expression pattern, thus providing a mechanism to tune the threshold and gene expression program triggered by stress signals. Indeed, we found that the level of glucose repression influences the bimodal expression behavior (Fig. 2). Cells grown in high glucose (2.0%) strongly repress their stress genes, thereby setting a threshold for the transition to an active transcriptional state upon salt stress to approximately 0.1 M NaCl. In low glucose medium (0.05%), glucose repression is alleviated, leading to a lowering of the expression threshold to 0.05 M NaCl, implying that a shorter window of Hog1 activity is sufficient to activate transcription under these conditions. Recent findings revealed that MAPK pathways possess a constant basal activity. The threshold set by the stepwise gene activation mechanism thereby prevents spurious expression of these genes under normal growth conditions. The comparison between high and low glucose expression behaviors implies that cells grown in less favorable nutrient conditions are more prone to express their stress response genes, thus revealing a physiological cross-talk between growth conditions and stress resistance.

Interestingly many stress response pathways share the same features responsible for bimodal gene expression in the HOG pathway. For example, heat- and oxidative stress conditions transiently activate signaling pathway, culminating in profound changes in gene expression dependent on the transcription factor Msn2. Analogous to osmotic stress, the expression of an Msn2-dependent fluorescent reporter is bimodal, with the population is split in expressing and non-expressing cells at low stress levels. What may be the physiological advantage provided by this bimodal expression mechanism? Although expression of stress genes is not required for the rapid adaptation response, transcription and new protein synthesis clearly improves long-term survival and resistance to future stress conditions of various kinds. We thus speculate that the large intrinsic noise in the expression of stress genes might lead to a large phenotypic variability in the stressed population, which may increase the chances of individual cells to survive subsequent stress events. While additional experiments are needed to test this intriguing hypothesis, these quantitative single cell measurements revealed important new insights into underlying signaling kinetics and the dynamic process of gene regulation in response to various stress conditions, which may also serve as a paradigm to understand other signaling pathways.

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