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Invited Review

Conditional Response to Stress in Yeast

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Summary. All living cells respond to sudden, adverse changes in their environment by evoking a stress response. Here we focus mainly on the response of the model eukaryotic organism *Saccharomyces cerevisiae* (baker's yeast) to an increase in external osmolarity. We summarize data demonstrating that stress responses largely depend on the existing environmental growth conditions. Nutrients and other medium parameters such as external *pH* determine how yeast cells sense osmotic stress circumstances and subsequently elicit adaptive responses. It is likely that the composition of cell wall and plasma membrane plays an important role in this process.

Keywords. Yeast; Stress response; Growth control; High osmolarity; Environmental conditions.

Introduction

A vital property of living cells is their ability to cope with adverse environmental conditions. The classical example of the underlying molecular mechanisms, commonly designated as stress response, is the heat shock response. The heat shock response has first been described in the fruitfly *Drosophila melanogaster* in the early sixties, and has been investigated in the model eukaryote *Saccharomyces cerevisiae* (baker's yeast) since the beginning of the seventies (reviewed in Refs. [1–5]). The response to increased temperature is characterized by massive changes in gene expression [6, 7]. At heat shock conditions, total protein synthesis is transiently reduced, whereas transcription of a subset of genes is strongly activated. These so-called *HSP*-genes (heat shock protein genes) encode proteins that assist in repair and recovery from, as well as adaptation to the potentially life-threatening conditions. Indeed, the most important aim of the cellular stress response is the acquisition of stress tolerance. This means that cells exposed to a mild stress become more

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In memoriam of *H. Ruis*

resistant to a second, more severe stress challenge. In the case of the heat shock response, this is – in part – brought about by increase in the concentration of heat shock proteins, which serve as molecular chaperones assisting in protein folding.

The classical heat shock-induced transcriptional response is mediated by the heat shock transcription factor Hsf1p, which binds to responsive elements, HSE, in the promoters of the target genes (see Refs. [1–5]). Only about ten years ago, it was recognized that apart from the classical heat shock response, yeast cells evoke a more general stress response, in order to protect against and build up tolerance to a wide variety of stress circumstances. In the group of *H. Ruis* it was first firmly established that apart from the Hsf1-HSE system, in yeast yet another system of stress-induced transcriptional regulation is operating, which is mediated by the transcription factors Msn2p and Msn4p and their cognate promoter element STRE [8, 9]. The general stress response can be triggered by different external stress agents, *viz.* exposure to heat, increases in osmolarity, exposure to salt, reactive oxygen species, heavy metals or acids, or starvation from essential nutrients. It is believed, though still not experimentally proven, that the occurrence of a shared response underlies the phenomenon of cross protection. This means that cells exposed to heat shock also become partially resistant to, for instance, hyper-osmotic shock, *e.g.* Refs. [1, 10]. Since the discovery of the general stress response, the picture of the stress response of *Saccharomyces* can be drawn as follows (see Fig. 1). Upon a specific stress challenge, both common and specific responses are elicited. The common part consists of the general stress response, which renders cells more stress resistant. Below we will review why the general stress response can be considered to relate to growth control as the other side of the same coin. The general stress response is mediated by Msn2p and Msn4p, which upon stress exposure become competent to activate transcription of the general stress responsive (STRE-driven) genes [9, 11–15]. Note that in the operational definition of the general stress response only changes in gene expression are implicated. Deletion of both *MSN2* and *MSN4* results in increased (severe) stress sensitivity with concomitant loss of stress-responsive gene expression [9], but whether also cross-protection phenomena are lost, is still elusive. Analyses of cellular levels of *mRNA*, for instance by means of microarrays, have revealed that

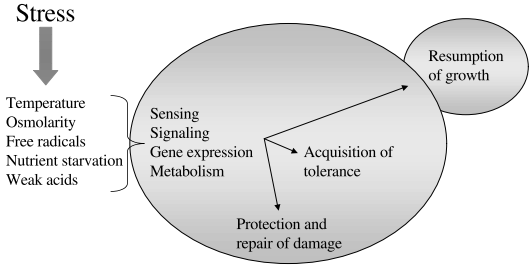

Fig. 1. General scheme illustrating the main principles of yeast stress responses; stress conditions (of different kinds, as indicated) are sensed by yeast cells and induce – both specific and general – signal transduction pathways leading to changes in gene expression and metabolism; the aim of the responses is to repair cellular damage, to protect cellular constituents, and to acquire stress resistance; after the onset of adaptation mechanisms, growth (which is transiently arrested after exposure of cells to stress) in general is resumed; stress control and regulation of growth are tightly interrelated

transcription of a few hundreds of yeast genes is enhanced (to different extents) after exposure to stress [6, 7, 16, 17]. Among these genes are of course the heat shock genes but, for instance, also genes involved in the biosynthesis of trehalose, a universal stress protectant. Besides the common response to many types of stress, specific stress response mechanisms are triggered. In this review we will mainly focus on hyper-osmotic stress. In this case the purpose of the specific response is the accumulation of the compatible solute glycerol (see below, for a recent review see Ref. [18]). For the oxidative stress response the production of anti-oxidant enzymes like peroxidase and superoxide dismutase, as well as radical scavengers such as glutathione and thioredoxin is important [19]. Heat shock conditions demand for adaptations in the composition of the yeast cell wall [20, 21]. The specific stress responses, thus, are characterized by molecular mechanisms that also under normal, non-stress, conditions form part of cellular homeostasis.

Stress Response versus Growth Control–Effect of Nutritional Conditions

For quite some time it is known that *cAMP*-dependent protein kinase A (PKA) activity is an important determinant of stress resistance in yeast. In studies using many PKA-mutants (with constitutively high or low PKA activity) it was shown that rapidly proliferating yeast cells, that most likely have a high level of PKA activity, display low stress resistance, *e.g.* Ref. [22]. On the other hand, cells having low PKA activity, for instance those growing on non-fermentable carbon sources or those approaching the stationary phase of growth, are stress tolerant [23, 24]. Thus, although it has been impossible to directly measure PKA activity *in vivo*, it seems a critical parameter of both growth control and stress response. It is likely that the relationship between PKA activity and stress resistance is mainly based on the regulation of Msn2p and Msn4p [13, 25–27]. As studied in *H. Ruis*' group these transcription factors are modified in a PKA-dependent manner, which determines their cellular localization (see Fig. 2) [9, 15]. At conditions of low PKA activity these factors are in the nucleus and promote transcription of stress-responsive genes, whereas at high PKA levels they are localized to the cytoplasm. Intriguingly, one of the genes which is activated via Msn2p/Msn4p is *YAK1*, encoding a protein kinase which antagonizes PKA activity [28–30]. An additional important parameter is trehalose. Also trehalose biosynthesis genes are induced in a Msn2p/Msn4p dependent manner, which forms another clue with respect to the general stress resistance of yeast at low PKA conditions.

In a recent study from *H. Ruis*' group the domain of Msn2p that governs nuclear translocation upon acute glucose starvation has been identified [15]. Strikingly, this domain did not drive nuclear routing by other types of stress, suggesting a fundamental difference in stress-induced signaling between glucose starvation and other stress conditions. PKA was shown to phosphorylate directly the respective Msn2p domain, hence inhibiting nuclear import [15]. Acute glucose starvation was inferred to lead to down-regulation of PKA and, hence, dephosphorylation of the Msn2p domain, but other types of stress had no effect.

It is as yet unclear whether, and if so, how stress conditions may influence the activity of PKA (see Fig. 2). Also the level of *cAMP* does not seem to be affected

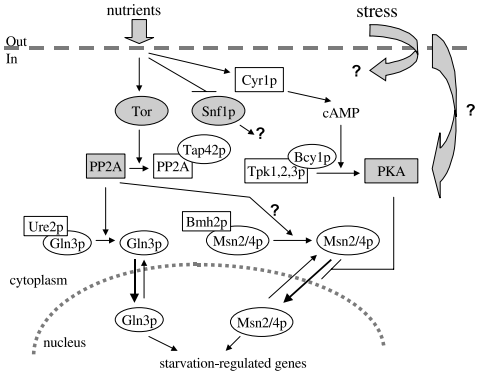

Fig. 2. Main features of nutrient-induced signal transduction; details are described in the text; basic principle is that nutrient sensing (and/or transport) triggers activity of cytoplasmic protein-modifying enzymes, *viz.* kinases or phosphatases, which in their turn regulate activity of specific transcription factors; the latter occurs by phosphorylating them directly or releasing them from their cytosolic anchoring proteins, thereby promoting the translocation of these transcription factors to the nucleus; once in the nucleus the transcription factors are able to stimulate transcription of nutrient starvation-specific genes; it is as yet elusive whether stress conditions interfere with the proper sensing of or direct signaling by nutrients, or affect activity of kinases or phosphatases involved in nutrient signal transduction

by stress challenges. It may well be that another common denominator of stress conditions exists which is distinct from PKA. An interesting mechanism through which PKA activity may be regulated or targeted to specific cellular substrates is by modification of its regulatory subunit Bcy1p [31]. It has been demonstrated that the cellular localization of Bcy1p is controlled by Yak1p kinase in the case of glucose depletion conditions and by Mck1p kinase in the case of heat stress (*G. Griffioen*, University Leuven, Belgium, personal communication).

In agreement with the opposite correlation between PKA and stress resistance, growth medium conditions have a major effect on yeast stress responses. In general, the presence of glucose (as yeast's favorite carbon source) represses the stress response. On non-fermentable carbon sources, yeast exhibits stress tolerance, which – at least partly – can be explained by derepression of stress-responsive genes [24]. Also the presence or absence of a nitrogen source affects the yeast stress response [22, 32]. The interplay of nutritional conditions and stress signaling has become manifest in the pattern of transcription of stress-responsive genes (see Fig. 3). For example, we have analyzed transcriptional regulation of *HSP12* (as a nice monitor of stress conditions, [26]) after a stress challenge on different culture media. During steady state growth conditions, stress-responsive gene expression is repressed in the presence of glucose and derepressed when a non-fermentable carbon source is present. The opposite holds true for ribosomal protein genes as monitor of growth-related genes [33]. Typical results are presented in Fig. 3A. On the other hand, the immediate response to stress is much stronger in yeast cultured in fermentable conditions. The typical results presented in Fig. 3B demonstrate that both the kinetics and the amplitude of the response are strongly carbon source-dependent [24]. Apparently, under glucose growth conditions the stress-responsiveness of the cell is much greater than in non-fermentable conditions. In addition, the

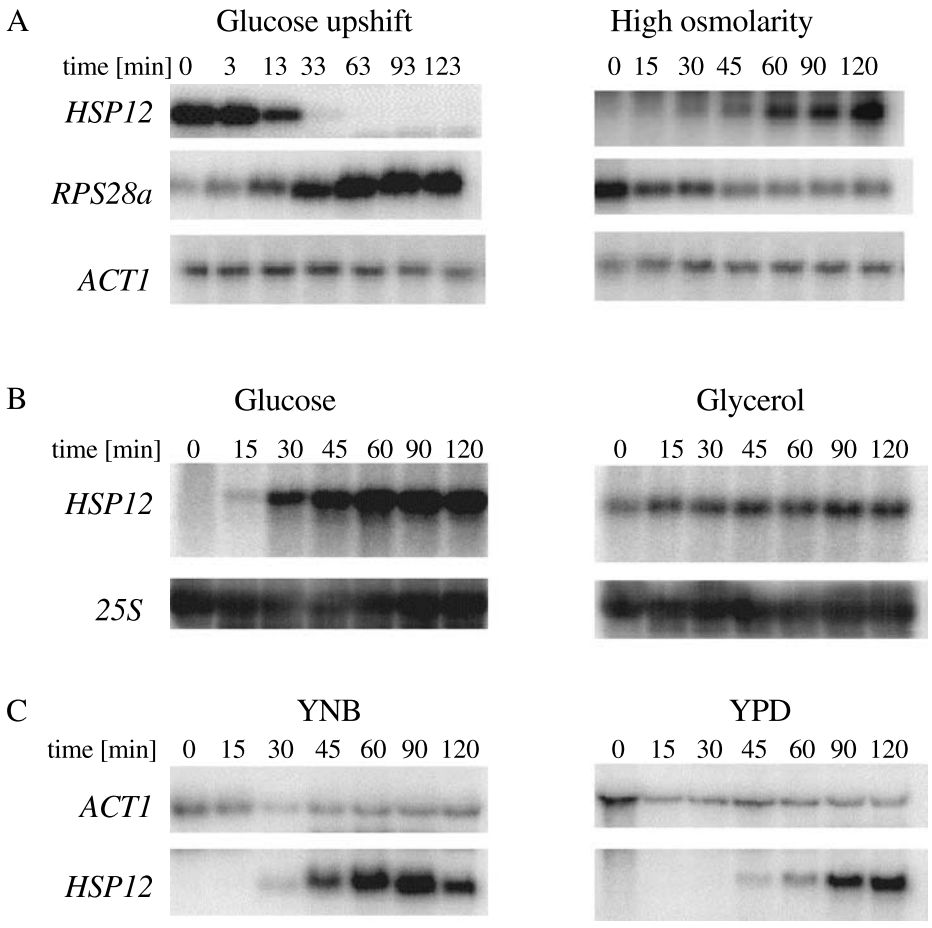

Fig. 3. Typical examples showing the effect of nutrient conditions on stress-responsive transcriptional regulation; A. Inverse regulation of stress-responsive genes (exemplified by the gene encoding the small heat shock protein gene *HSP12*) and growth-related genes (ribosomal protein gene *RPS28a* being an example); the Figures show the results of typical Northern blot analyses of *mRNA* levels (actin *mRNA* or 25S *rRNA* used as an internal gel loading control); left panel: yeast cells were grown on a medium with a non-fermentable carbon source (glycerol) and glucose was added at time = 0 (glucose upshift); the glucose addition leads to a rapid and dramatic repression of *HSP12* gene expression and, on the contrary, a considerable increase in *RPS28a* gene expression; right panel: yeast cells were grown in glucose-containing medium without osmotic stress and at time = 0 salt is added; the salt addition leads to a dramatic increase in *HSP12* gene expression and, in contrast, a decrease in *RPS28a mRNA* levels (courtesy to *E. de Groot*); B. Hyperosmotic stress-responsive transcriptional regulation of *HSP12* in yeast growing in a medium containing glucose (left panel) or glycerol (right panel) as carbon source; glucose-grown yeast cells display a much stronger response to the imposed osmotic stress (exposure to 0.7 *M* NaCl) than glycerol-grown cells; on glycerol-containing medium *HSP12* is already expressed in the absence of stress and an osmotic stress challenge gives rise to only a moderate gene activation; C. Hyper-osmotic stress-responsive (1 *M* NaCl) transcriptional regulation of *HSP12* in yeast growing in poor (YNB, yeast nitrogen based) medium (left panel) and rich (YPD) medium (right panel); clearly the kinetics and amplitude of the response are influenced by the type of culture medium

pattern of stress-induced transcriptional regulation differs significantly when cells are cultured in YPD (rich medium) as compared to YNB (yeast nitrogen based medium) – see Fig. 3C. The molecular control mechanism behind this difference is unknown. Few data are available as yet with respect to other stress-induced 'read-outs' such as MAP kinase phosphorylation (see below). The data summarized above clearly indicate that nutrients have a strong effect on the response of yeast to stress and are a major determinant in the balance between growth control and stress regulation.

With respect to control of yeast cell growth, a central but as yet rather elusive role is played by Tor kinase (see Fig. 2, reviewed in Ref. [34]). Tor1p and Tor2p are protein kinases showing homology to phosphatidyl-inositol kinase (PIK-related kinase). Tor depletion, which can be characteristically brought about by treatment of yeast with the immunosuppressing drug rapamycin, causes a starvation response: repression of translation initiation and arrest of cell growth at G1. Tor kinase is active under optimal growth conditions. Glucose and amino acid sensors likely play a part in maintaining its activity. When a preferred carbon or nitrogen source in the medium is limiting, Tor kinase activity is inhibited which leads to activation of several downstream targets. First, the activity of several transcription factors is stimulated, since these are released from their cytoplasmic anchoring partners and translocate to the nucleus [35]. Gln3p and Gat1p, which are implicated in nitrogen catabolite repression, belong to these transcription factors and notably also Msn2p and Msn4p. Supposedly, Msn2/4p are retained in the cytoplasm by interaction with the 14-3-3-proteins Bmh1p and Bmh2p. Upon dephosphorylation of Bmh1/2p, Msn2/4p may translocate to the nucleus and, hence, promote transcription of STRE-controlled genes (see Fig. 2).

A possibly direct substrate of Tor kinase is protein phosphatase PP2A ([34], Fig. 2). PP2A activity is downregulated via Tor-mediated phosphorylation of Tap42p, the regulatory subunit of PP2A. Control of PP2A activity may also underlie (part of) the Tor-mediated transcriptional regulation mentioned above, and thus, most likely reflects a key function of Tor. Notably, *PPH22* (encoding one of the catalytic subunits of PP2A) overexpression leads to a 'high PKA-phenotype', which is, for instance, characterized by constitutive repression of stress-responsive genes. This finding suggests that a regulatory link may exist between this phosphatase and *PKA*. Recently, in a study of transcription factor Gln3p activation, the intriguing observation was made that Tor-mediated nitrogen signaling and Snf1p-mediated glucose signaling may converge while they have opposing effects on Gln3p activity [36]. Snf1p kinase plays a central role in glucose repression and it is tempting to speculate that it may also exert its function on the activity of Msn2/4p. Indeed, the *MSN2* and *MSN4* genes were first described as multicopy suppressors of a *snf1* mutant phenotype.

Whether or not stress conditions interfere with the Tor and/or Snf1 pathway – which would be an appealing model – remains to be established (Fig. 2). It is noteworthy that in the fission yeast *Schizosaccharomyces pombe*, the *tor1* deletion strain displayed sensitivity to different types of stress, including high osmolarity and high temperature [37]. Such analyses have not yet been reported for *Saccharomyces cerevisiae*.

Hyper-Osmotic Stress Response

When *Saccharomyces* is exposed to an increase in external osmolarity, cells shrink as a consequence of water loss. Depending on the level of osmotic shock, this may lead to an immediate growth arrest since a critical cell size is required for growth and division of yeast cells. In order to swell and regain turgor, cells start to accumulate glycerol as a compatible solute. The latter occurs via two mechanisms. First, the plasma membrane-localized glycerol transport facilitator Fps1p immediately closes, hence avoiding efflux of glycerol. Secondly, biosynthesis of glycerol is stimulated, amongst others by increase in the transcription of the corresponding genes. It goes beyond the scope of this review to describe and comment all details of the hyper-osmotic stress response. Extensive reviews have appeared recently [5, 18, 38]. We limit ourselves to the aspects that are relevant for the issue of this article and just summarize the main features.

The canonical view of a stress-signaling pathway is a series of molecular events, which starts with a sensor, proceeds with signal transduction cascade, and leads to the activation of a transcription factor. At the level of the yeast plasma membrane, one protein, Sln1p, has been found to act as osmosensor (see Fig. 4) [39]. The protein forms part of a signal sensor-receiver system reminiscent of the bacterial two-component systems. The molecular partners of Sln1p are the phospho-relay protein Ypd1p and the signal receiver Ssk1p. Ssk1p serves as

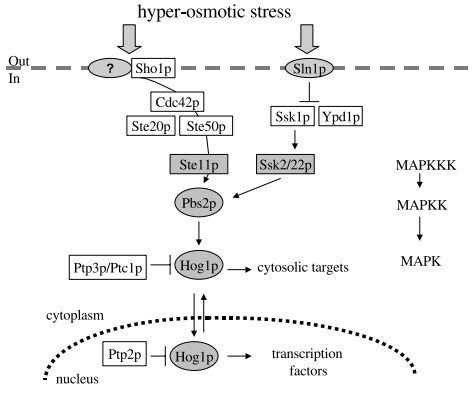

Fig. 4. Schematic overview of the hyper-osmotic stress-induced HOG MAP kinase pathway; details are described in the text; two input branches signaling into the HOG MAP kinase module can be distinguished; one is mediated by a signal receiver – response regulator complex consisting of Sln1p, Ypd1p, and Ssk1p; the other is mediated by an upstream protein complex consisting of (at least) Cdc42p, Ste20p, and Ste50p, which is recruited to the plasma membrane by Sho1p; the actual osmosensor in this case has not yet been identified; *via* the Sln1-branch MAPKKK Ssk2/Ssk22p is activated, which phosphorylates and activates MAPKK Pbs2p; the Sho1-branch activates MAPKKK Ste11p, which can also activate Pbs2p; Pbs2p is the dual specificity kinase that specifically phosphorylates MAP kinase Hog1p; upon phosphorylation, Hog1p translocates to the nucleus in order to contribute to the osmotic-stress induced gene activation; two known target transcription factors are Hot1p (a.o. implicated in *GPD1* – glycerol 3-P dehydrogenase – gene expression) and Sko1p (repressor of a.o. *ENA1* encoding the sodium pump); in addition, most likely Hog1p has several cytoplasmic functions; de-activation of the pathway is mediated via the action of the phosphatases Ptp2p, Ptp3p, and Ptc1p

upstream kinase of an osmo-specific signal transduction route, the HOG (high osmolarity glycerol) MAP kinase pathway [40]. The HOG pathway is composed by the MAPKKKs Ssk2p and Ssk22p, the dual-specificity MAPKK Pbs2p, and the MAPK Hog1p (see Fig. 4, for a review see Ref. [41]). Pbs2p is also activated by another upstream branch of the HOG pathway, which consists of the membrane protein Sho1p, the small G-protein Cdc42p, upstream kinases Ste50p and Ste20p, and the MAPKKK Ste11p [42–45]. It is unlikely that Sho1p has an osmostress-sensing function itself; it may rather serve to recruit the signaling complex to the plasma membrane. Indeed, expression of solely the cytoplasmic part of Sho1p fused to a plasma membrane-recruiting peptide appeared to convey full functionality [44]. Most likely recruitment of downstream signaling molecules occurs at the locations where the yeast cell is most vulnerable to osmotic pressure, *viz.* at the sites of bud emergence and mating projection. Once phosphorylated by Pbs2p, Hog1p is translocated to the nucleus [46, 47] where it meets its substrate proteins, for instance the transcriptional repressor Sko1p and the transcriptional activator Hot1p [48–50]. Evidence has been obtained that Hog1p associates with the transcription machinery and transcript profiling has elucidated the impact of Hog1p on osmostress-induced transcriptional regulation [51]. Recently it has become clear that Hog1p in addition has cytoplasmic targets. Among those is Rck2p, a protein kinase implicated in regulation of translation elongation [52, 53]. This data suggests the appealing model that, as part of the osmostress-induced re-setting of cellular metabolism, Hog1p temporarily inhibits protein biosynthesis.

Recently, evidence is accumulating that the HOG pathway plays an important role in maintenance of cellular integrity as well [54, 55]. In the past, two other MAP kinase signaling pathways have been implicated in control of cell integrity, *viz.* the PKC pathway, reviewed in Ref. [41], and the SVG pathway [56]. The PKC pathway is triggered by conditions of hypo-osmotic shock [57] and, moreover, by elevated temperature [20, 58]. The SVG pathway, for the major part consisting of components functioning in pseudohyphal growth control, is supposed to be triggered by cell wall defects [59]. We have found evidence supporting the idea that all three pathways, HOG, PKC, and SVG, are involved in control of cell wall formation, and hence, in maintenance of cellular integrity [55]. For instance, mutant cells defective in the HOG pathway in general display hypersensitivity to the cell wall degrading enzyme zymolyase and resistance to the chitin-binding drug calcofluor white [54, 55]. In addition, growth defects of certain HOG pathways mutants at increased osmolarity appeared to be suppressed by overexpression of genes involved in cell wall synthesis. We will discuss below how external conditions may influence the global coordinate regulation through the signaling routes implicated in control of cellular integrity.

Effect of Environmental Conditions on Stress Signaling

As expected on the basis of the role of the HOG MAP kinase pathway in the hyper-osmotic stress response, HOG pathway mutants display osmosensitive phenotypes – see Fig. 5A. Notably, however, we found that the osmosensitivity of the *hog1* mutant strain is strongly reduced at elevated temperature (*viz.* 37°C [55, 60], see Fig. 5A). This apparent temperature-remedial osmoresistance was

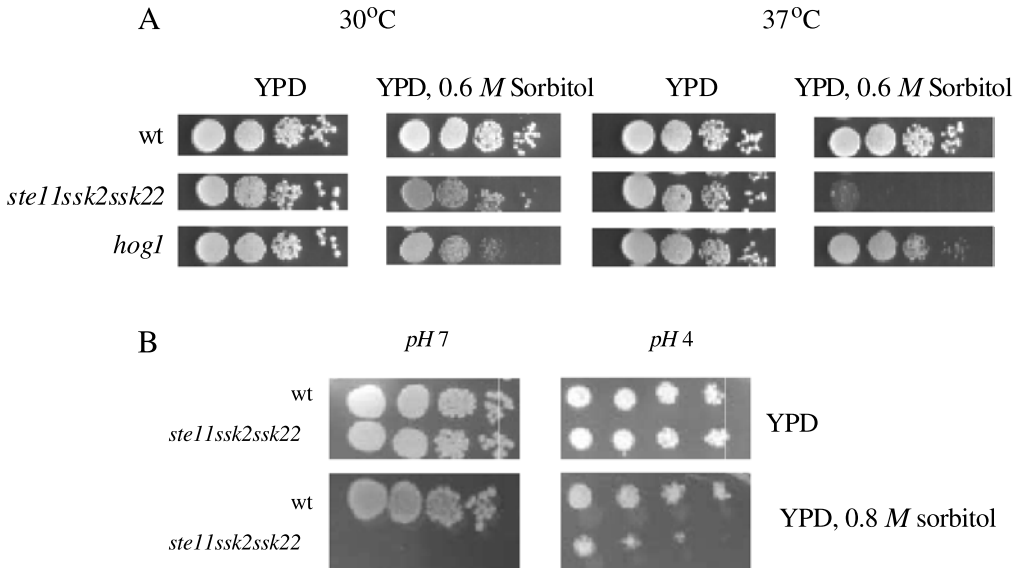

Fig. 5. Plate assays demonstrating the effect of environmental conditions on osmostress resistance of yeast; A. Wild type yeast cells as well as several HOG pathway mutant strains were grown on YPD medium containing 0.6 *M* sorbitol at 30°C (left panel); the figures show drop assays of a 1:10 dilution series of the respective yeast culture; HOG pathway mutants display osmosensitivity; the right panel demonstrates that at the elevated temperature of 37°C osmosensitivity in general is strongly diminished in the *hog1* strain but exacerbated in the *ste11ssk2ssk22* mutant; B. Wild type yeast cells as well as the *ste11ssk2ssk22* mutant strains were grown at 37°C on YPD medium containing 0.8 *M* sorbitol at *pH* 7 and 4; the hyper-osmosensitivity of the *ste11ssk2ssk22* is reduced at acidic external *pH* as compared to neutral *pH*

correlated with a higher basal content of glycerol. The molecular mechanism underlying the higher glycerol levels in HOG pathway mutants at 37°C has not been elucidated. It is unlikely due to enhanced synthesis of glycerol at increased temperature. After a heat shock the glycerol-biosynthesis genes do show elevated expression, but this is a transient phenomenon. Rather an appealing possibility is that less glycerol is exported from the cell. This may reflect a different action of the glycerol facilitator Fps1p, possibly as a consequence of the altered structure of the cell wall in HOG pathway mutants. In contrast to the *hog1* mutant, the *ste11ssk2ssk22* strain displayed hypersensitivity at elevated temperature (Fig. 5A, [55]). Genes involved in cell wall organization were isolated as suppressors of the hyper-osmosensitivity at elevated temperature, indicative of common regulatory output for HOG, PKC, and SVG signaling.

Further evidence for the interplay of environmental conditions is the finding that HOG signaling in wild type yeast cells is reduced at elevated growth temperature. As is shown in Fig. 6A, Hog1p phosphorylation is even more transient at 37°C than at 24°C. Obviously yeast cells are sensing osmotic stress to a different extent, depending on the growth temperature.

We recently made an additional striking observation with respect to the influence of external *pH* on HOG pathway signaling. As can be concluded from the results presented in Fig. 6B, signaling through the HOG pathway is dramatically

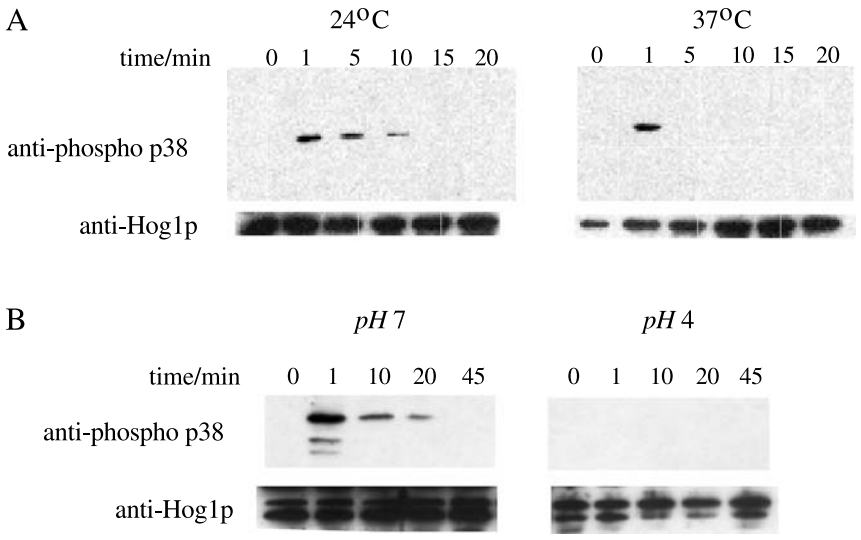

Fig. 6. Osmotic stress-induced Hog1p-phosphorylation depends on environmental conditions; the figures show the results of Western blot analyses, in which specific antibodies have been used to detect dually-phosphorylated Hog1p and total Hog1p, respectively; in A. the characteristically transient nature of Hog1p-phosphorylation after an osmotic challenge of cells grown at 24°C is shown (left panel); when yeast was grown at 37°C, the temporary activation of Hog1p is even more notable; in B. the results of a similar experiment using cells grown at an external *pH* of 7.0 and 4.0 are shown; strikingly, no Hog1p phosphorylation could be detected at lower *pH* (courtesy to *E. Real*)

reduced after adjusting the *pH* of the growth medium to 4.0. Interestingly, also the hypersensitive phenotype of the *ste11ssk2ssk22* mutant at elevated temperature is suppressed by lower *pH* (see Fig. 5B). In addition to these observations, previously also in *H. Ruis*' group the dramatic effect of medium *pH* on stress-induced gene expression was observed [61].

Possibly this pH-related phenomenon reflects altered sensing of the osmotic stress at the level of cell wall or plasma membrane. Indeed, it has been found that low environmental *pH* strongly affects the molecular organization of the yeast cell wall [62]. This was concluded on the basis of the induced resistance to β-1,3-glucanase treatments and the modified incorporation of cell wall proteins. As was demonstrated by microarray analyses, shifting the *pH* of a yeast culture from 5.5 to 3.5 resulted in major changes in gene expression, quite similar to changes induced by hyperosmotic shock.

In *H. Ruis*' group evidence has been obtained that at least part of the general stress signal is generated at the level of the plasma membrane [61]. The use of several drugs and detergents or means to reduce the activity of plasma membrane *ATP*ase (by mutations or inhibitors) demonstrated that defects at the level of plasma membrane permeability or potential results in nuclear translocation of Msn2p/Msn4p and, hence, activation of general stress-responsive genes. It is unknown whether this is a evidence that sensing of stress – at least partly – takes place at the plasma membrane or that it is an indirect effect, *e.g. via* PKA activity.

These findings emphasize that the osmostress response of yeast is largely dependent on the general status of the cell, which in its turn is a reflection of the

surrounding environment. The picture arises that osmostress resistance of yeast on the one hand is linked to the general condition of the cell with respect to PKA activity, and *e.g.* the consequent levels of trehalose. On the other hand it is related to the sensing-competent state of the cell, which in its turn is determined by the structure of the cell wall and/or plasma membrane. Most likely the intracellular glycerol level is an additional important parameter for the sensing capacity of yeast cells. Future studies have to reveal the molecular mechanism by which the different parameters integrate in order to determine the stress-sensing ability of yeast.

Recent studies have clearly demonstrated that yeast cells adapt to environmental changes by defined programs of gene expression. Therefore, detailed expression profiling may help to unravel the integration of the molecular signals elicited by the many external factors yeast normally has to deal with.

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