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Signalling Stress in Yeast

Juan Carlos Igual[1] *and Francisco Estruch*[1,2]*

[1]Departamento de Bioquímica y Biología Molecular, Universitat de València

[2] Departamento de Biotecnología, IATA (CSIC), Apdo. Correos 73, 46100 Burjassot (Valencia), Spain

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Summary

The response to environmental conditions involves a complex system of signal transduction pathways. These pathways allow the sensing of the external alteration and the transmission of the signal from the surface of the cell to the nucleus. In this way, the stress signals are converted into changes in gene expression. The proteins synthesized under these conditions allow damaged cells to be repaired and protected against further exposure to stress. In this review we analyze the stress-activated signal pathways in the yeast *Saccharomyces cerevisiae*. We will describe the progress made in recent years in studies about how the stress signal is sensed and transduced to the nucleus, how transcription factors are activated and which genes are induced.

Key words: yeast, stress, heat-shock, nutrient signalling, cell integrity, transcription

Introduction

To survive from the environmental challenges, cells have developed mechanisms to sense the external stimuli and convert them into appropriate cellular responses. Many of these responses involve changes in gene expression and require signal transduction pathways to communicate the sensor, located on the surface of the cell or in the cytoplasm, with the transcriptional machinery, located into the nucleus. Stress responses are particularly important in the highly variable environment of microorganisms, where conditions such as temperature, osmolarity or nutrient availability are far from being constant. Among them, the yeast *Saccharomyces cerevisiae*, an organism that permits genetic approaches, has become a model to study at molecular level how eukaryotic cells respond to stress.

Among the different steps essential for stress-signals transduction, sensing is the least understood. A transmembrane histidine kinase, Sln1p, has been identified as sensor of external osmolarity in the HOG MAPK pathway (*1*, see below). However, for most pathways exactly how the stress signals are sensed and how the sensing component communicates with the next component of the pathway is poorly understood. Once any environmental alteration has been sensed, signals must be transmitted to the nucleus. A number of protein kinases have been involved in this process and in some pathways protein kinases appear organized in an evolutionary conserved module known as MAP kinase cascade (*2*). Finally, each pathway activates one or more transcription factors that regulate the expression of a characteristic set of genes within the nucleus. In some pathways, such the General Stress Response, the transcription factor accumulates in the nucleus following the imposition of stress (*3*). In others, nucleocytoplasmic translocation takes place earlier in the pathway and the transcription factor is activated inside the nucleus, as it occurs in the HOG pathway (*4,5*). Whatever the mechanism used, the activated transcription factor binds to specific *cis* elements in the promoter of stress-regulated genes and induces their transcription. A variety of stress-activated transcription factors have been identified, but their adscription to a specific signal pathway is

* Corresponding author; Phone: ++34 963 900 022; Fax: ++34 963 636 301; E-mail: francisco.estruch@uv.es

not always clear. The complexity at this level is considerable: some transcription factors can be activated through different pathways, equally there are pathways that operate through different factors.

Besides the multi-component pathways, there are also examples where the transcription factor is directly activated by the stress condition or in a way that involves only a few regulatory functions. Thus, the transcription factor Yap1 is regulated at the level of cellular localization, through a C-terminal cysteine-rich domain that acts directly as sensor of the redox state (*6*).

In this review we will analyse the stress-activated pathways that have been described in the yeast *S. cerevisiae*. Additionally, we will discuss the physiological role of these pathways in the light of the genes that each pathway regulates and how their products protect cells against the deleterious effect of stress.

The cAMP-PKA Pathway and the General Stress Response

In budding yeast, the cAMP-PKA pathway has been involved in the control of many cellular processes, including nutrient sensing, regulation of yeast cell proliferation, carbon storage and stress response (*7*,*8*). The role of the cAMP-PKA pathway as sensor of the nutritional status of the cell has been suggested by the phenotypes shown by mutants in this pathway. Those mutants with reduced pathway activity respond as if nutrient-limited, even in rich medium, and arrest at the same point of the cell cycle as cells starved for nutrients (*9*). This has been related to the requirement of cAMP for the transcription of G1 cyclins (*10*). On the other hand, mutants with overactive cAMP-PKA pathway fail to arrest in G1 upon nutrient starvation (*11*). These results have been interpreted in such a way that cells with an activated pathway are unable to respond to the loss of nutrients, fail to arrest in G1 and become stranded throughout the cell cycle as they run out of resources. However, further studies revealed that cells with an activated cAMP-PKA pathway do not continue to divide after nitrogen starvation, but are unable to complete their current cell cycle and arrest at different points (*12*). Therefore, the sensitive phenotype shown by these mutants could be the consequence of the improper arrest of the cell cycle in starvation conditions.

The components of the cAMP-PKA pathway (Fig. 1) have been extensively studied (reviewed in *8*). The cAMP is synthesized by the adenylate cyclase, encoded by the *CYR1/CDC35* gene and, as in most eukaryotic cells, increased intracellular cAMP concentrations activates the cAMP-dependent protein kinase (PKA) by binding to the regulatory subunit (encoded by the *BCY1* gene) and dissociating the catalytic subunits (encoded by *TPK1*, *TPK2* and *TPK3*). Recently, Reinders *et al*. (*13*) have identified the Rim15p kinase as a component of the cAMP-PKA pathway acting immediately downstream and under the negative regulation of PKA to control a broad range of adaptations in response to nutrient limitation, including transcriptional mechanisms (see below). The activation of adenylate cyclase can occur through different pathways, involving a G-protein coupled receptor (GPCR) system or the GTPases Ras1p and Ras2p. The GPCR would activate adenylate cyclase in response to glucose (*8*). The function of the Ras proteins might be related to the sensing and transmission of stress signals on the basis that the activity of the guanine nucleotide exchange factor for Ras, Cdc25p, is positively regulated by the Heat Shock Protein Ssa1p (a member of the Hsp70 family, see below) through a direct interaction (*14*). Therefore, it has been proposed that the accumulation of denatured proteins upon stress conditions would reduce the activity of the cAMP-PKA pathway by recruiting Hsp's and reducing their interaction (and thereby its positive effect) with Cdc25p.

The activation of PKA causes transient changes in several systems, including trehalose and glycogen metabolism, glycolysis and gluconeogenesis, which contain

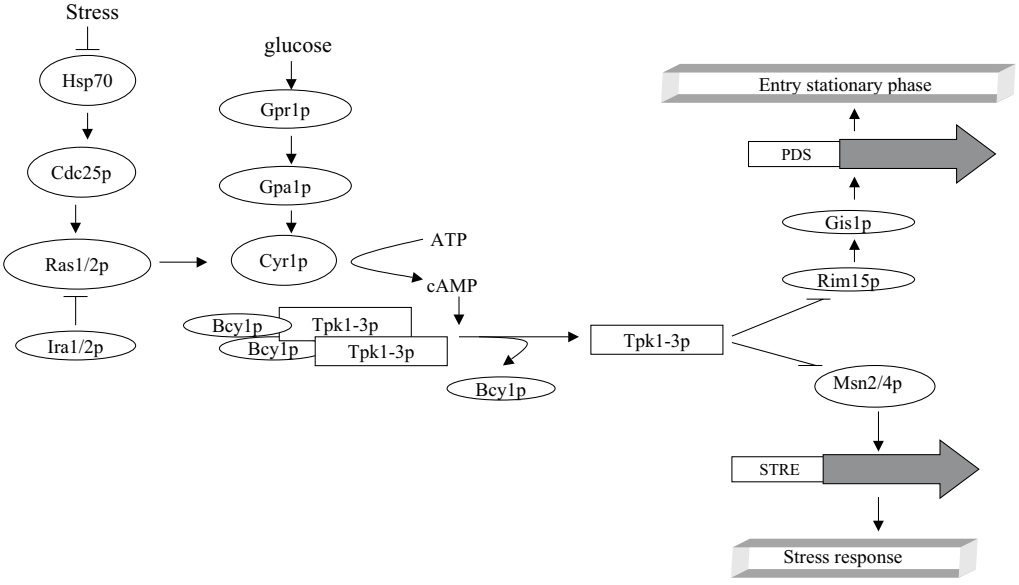

Fig. 1. The cAMP-PKA pathway and the control of the expression of STRE- and PDS-containing genes

components controlled by PKA-mediated phosphorylation (*8*). Moreover, basal and induced expression of some *HSP* genes was found to be enhanced in *ras2* mutants with low PKA activity and dramatically reduced in *bcy1* mutants with high constitutive PKA levels (*15,16*). The group of genes negatively regulated by the cAMP-PKA pathway are activated in response to very different stress conditions. According to this, a strong correlation has been observed between the activity of PKA and the extent of thermotolerance: cells with low PKA activity are remarkably thermotolerant whereas cells with high constitutive PKA are thermosensitive under all conditions (*15*). These observations suggest the existence of integrating mechanism, which is sensing and responding to different forms of stress: the *general stress response*. Additionally, a common *cis*-regulatory element has been identified in the promoter of many genes whose induction is mediated by different forms of stress (*17,18*). This element was designated STRE (by stress responsive element), and has the core consensus sequence AGGGG (*15*). STRE sequences have been identified in many stress-induced genes and a computer search of the entire yeast genome predicts as many as 186 potential STRE-regulated genes (*19*). This sequence is sufficient for the activation of a reporter gene by different types of stress, is functional in both orientations, and is negatively regulated by the cAMP-PKA pathway (*20*). Besides its negative regulation by the cAMP-PKA pathway, STREs have been involved in HOG-dependent transcriptional activation (see below).

In relation to the *trans*-acting factors involved in STRE-mediated genes expression, two zinc finger proteins, Msn2p and Msn4p, have been shown to specifically bind to this sequence (*21,22*). The *MSN2* gene was isolated as multicopy suppressor of the raffinose utilisation defect shown by mutants with a thermosensitive allele of the *SNF1* gene. A second similar zinc finger gene, *MSN4*, was isolated by sequence homology and shares with *MSN2* the capability to suppress the thermosensitive *snf1* mutation when overexpressed (*23*). However, Snf1p is not required for transcriptional induction mediated by STRE (*21*). The transcription factors Msn2p and Msn4p recognise and bind STREs both *in vitro* and *in vivo* (*3,21*). Footprinting analysis shows that the binding is enhanced by stress, coinciding with the nuclear accumulation of both factors (*3*). PKA pathway is involved in localization of Msn2p and Msn4p; there is an inverse correlation between PKA activity and nuclear localization, and mutations in the potential PKA modification sites of Msn2p lead to constitutive nuclear localization of this protein. Considering these results, a model of regulation in which stress induces nuclear import and PKA activity induces export by independent mechanisms has been suggested (*3*). However, translocation of Msn2p and Msn4p is not the only regulatory mechanism involved in STRE-mediated activation. Nuclear export of Msn2p and Msn4p depends on the product of the *MSN5* gene, encoding an export receptor that interacts with the small GTPase Ran and involved in different signalling pathways (*24,25*). In a *msn5* mutant Msn2p and Msn4p are nuclear localised even in non-stress conditions (*26*), but, in spite of this constitutive nuclear localization, regulation of STRE-dependent genes is not affected in *msn5* mutants (P. M. Alepuz and F. Estruch unpublished results), suggesting additional mechanism(s) to regulate Msn2p and Msn4p activity. Recently, Beck and Hall (*27*) have reported that rapamycin, acting through the TOR pathway, induces the nuclear accumulation of Msn2p and Msn4p. The effect of the rapamycin treatment is to release Msn2p and Msn4p from Bmh2p that retains these factors in the cytoplasm. Release of Msn2p and Msn4p from Bmh2p can be also induced in response to glucose withdrawal, although the effect of other stress conditions on this interaction has not been investigated. Bmh2p, and its homologue Bmh1p, are 14-3-3 protein required for Ras/MAPK cascade signalling during pseudohyphal development (*28*). The relationship between the TOR and the Ras/PKA pathway in controlling the nuclear localization of Msn2p and Msn4p is an important question that deserves further analysis.

Although one can imagine a scenario where Msn2p and Msn4p are activated by very different forms of stress and induce the transcription of a large battery of genes, the situation is not so simple. Treger *et al.* (*19*) have analysed the requeriment for Msn2p and Msn4p in the induction by heat shock of several STRE-containing genes. They have found that for some genes, such *PGM2*, heat-induction is completely abolished in a *msn2* single mutant. For most of them, the induction is reduced in the *msn2* single mutant and abolished in the *msn2 msn4* double mutant. They even have found a gene, *PDE2*, which heat-induction is not afected by the absence of Msn2p and only is defective in the double mutant strain. Moreover, the contribution of Msn2p and Msn4p in the activation also depends on the stress condition (M. Amorós and F. Estruch unpublished results). Finally, Msn2p and Msn4p are not required for osmotic stress induction of STRE, although the absolute level of transcription is severely reduced (*21,29*).

As it has been mentioned, due to the presence of STRE sequences in their promoter, many yeast genes are candidates for regulation through the General Stress Response pathway. The list includes genes involved in carbon metabolism, transporters, proteases and genes with protective functions against different types of stress, among them *HSP104*, *CTT1* and the trehalose metabolism genes *TPS1*, *TPS2*, *TPS3* and *TSL1* (*19,30*). In agreement with their role as main STRE transcription factors, *msn2msn4* double mutants show a higher sensitivity to different stresses, including carbon source starvation, heat shock and severe osmotic and oxidative stresses. On the other side, overexpression of *MSN2* and *MSN4* genes improves the resistance to starvation and thermal stresses (*21,31*). However, the phenotypes shown by the *msn2msn4* mutants are not as severe as one would expect considering the number of genes regulated through the general stress response. The redundance of regulatory elements on the promoter of genes with important roles in the stress response could explain the relatively mild phenotypes of the mutants. Moreover, the pleiotropic sensitivity is not observed in stationary mutant cells, despite that most of the proteins induced at the diauxic transition depends on Msn2/4p (*30*), suggesting the activation of Msn2/4-independent protective mechanisms in stationary phase cells. A candidate to activate these mechanisms is the protein kinase Rim15p that it has re-

cently been involved in the physiological adaptations necessary for proper entry into stationary phase (*13*). *rim15* mutants show a defective induction of thermotolerance and starvation resistance upon entry into stationary phase and are unable to induce the post-diauxic transcription of genes such as *HSP12*, *HSP26* and *SSA3*. Genetic analysis has placed Rim15p immediately downstream and under the negative control of cAMP (Fig. 1) (*13*). The possibility that Rim15p is involved in the PKA regulation of Msn2/4p function remains to be investigated. Another gene whose expression requires Msn2p and Msn4p is *YAK1*. This gene encodes a protein kinase whose loss confers slow growth to a strain deleted for *TPK1*, *TPK2* and *TPK3*, the redundant genes for the catalytic subunit of PKA (*32*). Thus, Yak1p kinase appears to negatively regulate cellular processes that are essential for growth and are under PKA control. Interestingly, PKA activity is largely dispensable in a strain lacking Msn2p and Msn4p, suggesting a model in which the main essential function of PKA would be to inhibit the expression or function of growth inhibitory genes, such as *YAK1*, under the control of Msn2/4p (*32*).

A variety of evidence supports the existence of PKA-dependent transcriptional mechanisms non-mediated by STRE sequences. Another *cis* regulatory element called PDS (post diauxic shift) has been identified in the promoter of the *SSA3* gene. This element, with a sequence (T/AAGGGA) resembling the STRE, is also regulated by the cAMP-PKA pathway and is responsible for the induction of *SSA3* late in the growth curve (*33*). However, both elements seem to be regulated in a different way since PDS is only able to cause a marginal increase in expression in response to heat shock (*33*) and is not affected by mutations in transcription factors involved in STRE-driven gene expression (*21*, M. Amorós and F. Estruch unpublished results). Recently, a zinc finger transcription factor, Gis1p, that acts in the cAMP-PKA pathway downstream of Rim15p has been identified (*34*). Transcriptional analysis suggests that Gis1p controls activation through the PDS element in response to nutritional limitation.

The Tor Pathway: Nutrient Sensing and Growth

Tor proteins are emerging as key regulators in the control of cell growth in response to nutrients (*35,36*). They were identified as proteins in which mutations could confer resistance to the toxic effects of the immunosupressant rapamycin (Tor: 'target of rapamycin') (*37*). Tor proteins contain a region in the carboxyl-terminus with homology to phosphatidylinositol kinases. Although evidence for lipid substrates has been presented (*38,39*), the essential activity of Tor is probably as protein kinases (*40,41*). There are two related Tor proteins in *S. cerevisiae*, denominated Tor1p and Tor2p (*38,42,43*). Both Tor1p and Tor2p share an essential function in promoting growth. Tor2p, but not Tor1p, has an additional function regulating actin cytoskeleton dynamics through the PKC-pathway (see below) (*44,45*).

The growth-promoting function of the Tor proteins is regulated by nutrients: when nutrients are available Tor activity stimulates growth; by contrast, limitations in nutrients led to the inactivation of Tor signalling and cessation of growth. The way Tor activity is regulated by nutrients is beginning to be understood. In this respect, rapamycin is being an invaluable tool in elucidating this and other mechanisms involved in Tor signalling. Rapamycin binds with high affinity to a prolyl isomerase (FKBP or Rbp1p). This complex then binds to a region of Tor proteins adjacent to the catalytic domain, resulting in inhibition of Tor function (*43,46*). It is noteworthy that while rapamycin inhibits the promoting growth function shared by Tor1p and Tor2p, does not affect Tor2p-dependent actin organization function (*43*). This suggests that rapamacyn-Rbp1p complex does not inhibit kinase activity but rather blocks Tor access to those substrates involved in promoting growth. Finally, binding of the rapamycin-Rbp1p complex seems to be regulated by the phosphorylated state of the Tor protein (*36*). Nevertheless, in spite of increased knowledge of Tor regulation, the endogenous ligand controlling the binding of Rbp1p to Tor in response to nutrient availability has not yet been found.

Tor function is mainly mediated by regulation of protein phosphatase 2A activity (Fig. 2). Two closely related genes, *PPH21* and *PPH22*, redundantly encode the major PP2A catalytic subunit. In addition, yeast contains a 2A-related phosphatase encoded by *SIT4*. Both Pph21/22 and Sit4p associates with different regulatory subunits to constitute active phosphatase complexes. Tap42p, an essential protein, links Tor to the PP2A activity (*47*). In growing cells, Tap42p associates independently with both Pph21p/Pph22p and Sit4p. On the contrary, cessation of growth in stationary phase or in the presence of rapamycin correlates with dissociation of Tap42p from both phosphatases. These observations, together with the finding that a mutant allele of Tap42p confers rapamycin resistance, suggest that Tor proteins promotes association of Tap42p with the two phosphatases catalytic subunits, and that this is the key step in Tor signalling. Recently, it was demonstrated that Tor phosphorylates Tap42p and that phosphorylated Tap42p binds to the phosphatase catalytic subunits (*41*). However, the consequences of the interaction of Tap42p with Pph21/22p and Sit4p are not clear. It has been shown that, at least in the case of Pph21/22p, binding of Tap42p and the regulatory subunits required to form an active phosphatase complex are mutually exclusive (*41*). However, a simple picture in which Tor functions by just sequestering phosphatases catalytic subunits via association with Tap42p cannot account for all experimental data: for instance, *pph21pph22* mutants are not resistant to rapamycin and Tap42p are in a limiting amount compared to Pph21/22p. The most likely scenario is that association with Tap42p convert PP2A into an anti-phosphatase, which positively acts protecting specific phosphorylated proteins from being dephosphorylated (see *41*).

Cell growth is defined as accumulation of mass, basically by general protein synthesis. It has been shown that Tor proteins stimulate protein synthesis, and thus growth, by two means. First, Tor1p and Tor2p activate translation initiation (*48*). Mutants defective for both *TOR1* and *TOR2* genes, or cell treated with rapamycin, exhibit a severe decrease in translation initiation, and cease proliferation arresting in the G1 phase of the cell

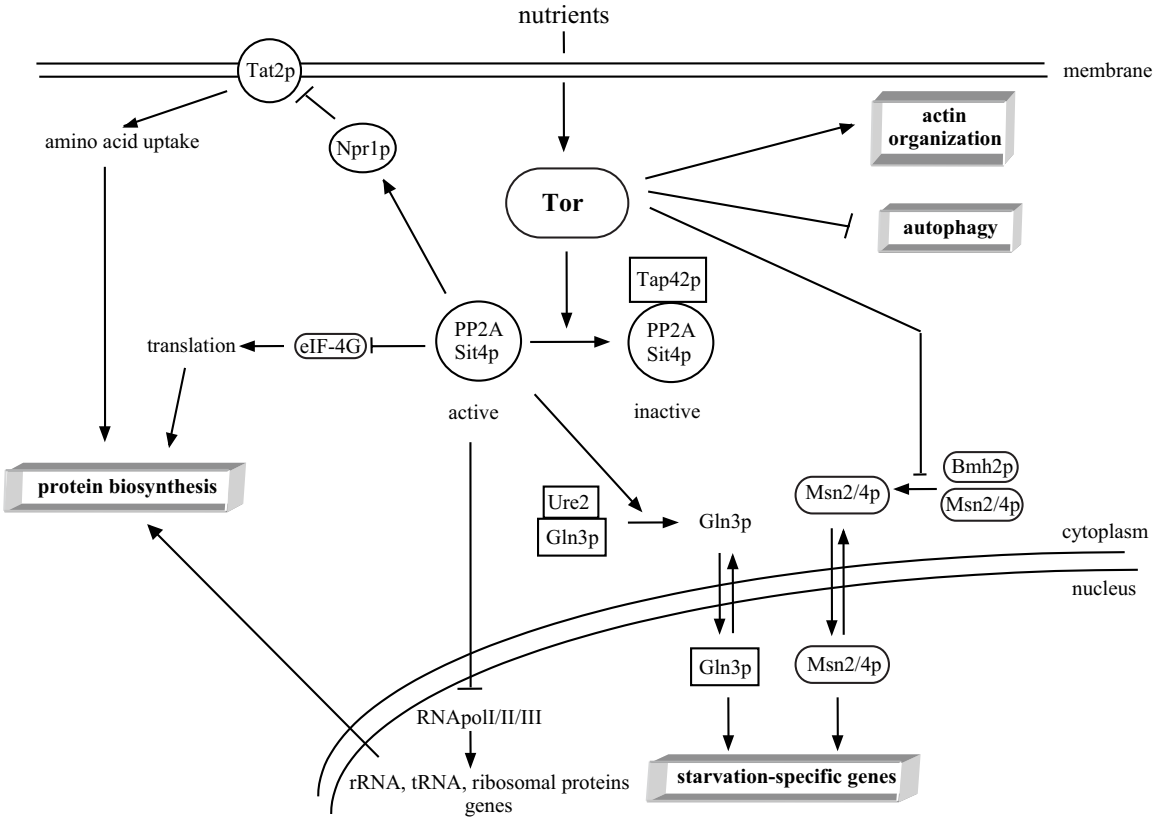

Fig. 2. The Tor pathway

cycle. This arrest is a consequence of the translation defect and probably reflects the fact that the highly unstable G1 cyclins are among the first proteins a cell misses in the absence of new protein synthesis. Tor control of translation is mediated by the regulation of the stability of the translation initiation factor eIF-4G (*49*), which serves as a scaffold for the assembly of the translational initiation complex. It has been shown that rapamycin or nutrient deprivation induces degradation of eIF-4G, and that expressing a rapamycin-resistant form of Tor1p can block this degradation. These results indicate that Tor functions to increase eIF-4G stability, thus positively regulating translational initiation. In addition to controlling initiation of translation, Tor also affects protein biosynthesis by regulating the synthesis of rRNA and tRNA (*50*). Rapamycin inhibits the transcriptional activity of RNApol I and RNApol III, which transcribe the large rRNA genes and the 5S-RNA/tRNA genes respectively. Indeed, RNApol I and RNApol III transcription was shown to be Tor-dependent. Moreover, Tor also promotes ribosome biogenesis by activating transcription of ribosomal protein genes (*51*). Thus, Tor proteins emerge as the master controllers of the protein biosynthetic activity, and consequently of the growth status, of the cell. This central role of Tor proteins in the regulation of protein biosynthesis converts them in appropriate targets for the starvation response triggered by nutrient limitations. If raw materials are limited, it is in the best interest of the cell to conserve energy and to avoid embarking on expensive anabolic processes such as protein synthesis and transcription of rRNA and tRNA genes (nearly 80 % of the cell transcriptional capacity). This will be accomplished by inactivation of Tor proteins.

Other aspects of the starvation response are also mediated by regulation of Tor activity. As a part of the starvation response, there is a severe decrease in the amino acid import. Tor controls amino acid uptake by increasing the stability of the high affinity tryptophan permease Tat2p (*52*). Destruction of Tat2p requires the dephosphorylation and consequent activation of the Npr1p kinase. Tor activity maintains Npr1 in a phosphorylated inactive state and thus stabilizes Tat2p. Because the inactivation of Npr1p by Tor is mediated by Tap42p, it is tempting to speculate that PP2A directly dephosphorylates Npr1 and that Tor-mediated association of Tap42 with PP2A directly inhibits this step. However, evidence for this hypothesis is elusive.

Starvation also induces bulk protein degradation through a process called autophagy. It involves sequestration of a portion of cytoplasm by an invaginated membrane structure, referred to as the autophagosome, which then fuses with endosomal or lysosomal membranes, resulting eventually in degradation of cytoplasmic materials. This degradation can generate amino acids and nucleotides, which can be used for energy. In yeast, rapamycin induces autophagy, suggesting that Tor acts as a negative regulator of autophagy. This was confirmed by the observation that a temperature-sensitive *tor* mutant strain induced autophagy at the nonpermisive temperature (*53*). It has been proposed that Tor probably acts negatively on Apg proteins, which control

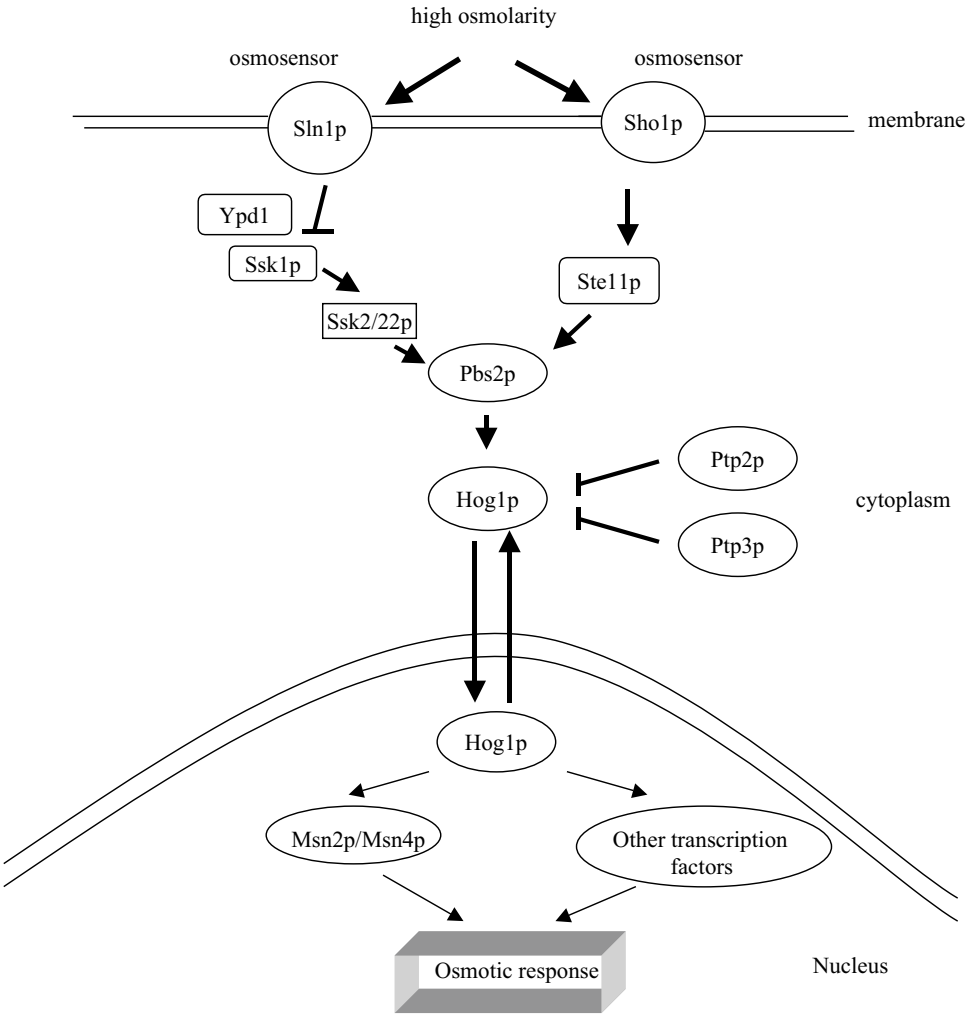

Fig. 3. The HOG pathway

the autophagy process, although the molecular mechanism underlining this control is unknown.

Finally, adaptation to starvation conditions also implies induction of transcription of specific genes. Recently, it was shown that Tor controls global repression of starvation-specific genes during growth in rich medium (*54,55*). Tor represses transcription of genes expressed upon nitrogen limitation by preventing nuclear localization of the transcription factor Gln3p (*27*). Gln3p is sequestered in the cytoplasm by association with Ure2p. Only phosphorylated Gln3p binds to Ure2p. Tor functions maintaining Gln3p in the phosphorylated state through association of Tap42p to the protein phosphatase Sit4p. On the other hand, Tor inhibits expression of carbon-source-regulated genes during growth in rich medium by preventing nuclear localization of the transcription factors Msn2p and Msn4p (*27*). As said before, retention in the cytoplasm is in this case mediated by association of Msn2p and Msn4p to the 14-3-3 protein Bmh2p. Contrary to the regulation of Gln3p localization, Tap42p and Sit4p do not seem to be involved in this mechanism, suggesting that Tor controls Msn2p-Msn4p by a different effector pathway.

In conclusion, Tor signalling coordinates diverse processes related to growth and, in this way enables an integrated response to limitation of nutrients: inactivation of Tor inhibits protein synthesis at the same time that induces degradation of proteins and activates synthesis of specific proteins required to adapt to poor nutrient conditions. Probably in near future other processes involved in the starvation response will be shown to be controlled by Tor activity.

The HOG Pathway: a MAP Kinase Pathway Specific for the Osmotic Response

In mammals, the MAPK (mitogen activated protein kinase) pathways have been implicated in many biological programmes, including cell division, inflammation, apoptosis and stress responses. In yeast, at least five processes are regulated through MAPK pathways, including mating, pseudohyphal growth, cell integrity (see below), sporulation and the response to high osmolarity. The HOG pathway, unlike other mammal stress-activated MAPK pathways, appears to be only activated by an specific stress signal: the increase of osmolarity (revie-

wed in 2). This pathway contains a MAPK module (Fig. 3), which consists of a cascade of kinases that when activated, culminates in the phosphorylation and activation of Hog1p (High osmolarity glycerol) protein kinase. In this cascade the dual specificity serine/threonine tyrosine MAPKK that phosphorylates Hog1p is Pbs2p. Pbs2p is in turn phosphorylated and activated by the serine/threonine MAPKKKS Ssk2p and Ssk22p. The sensor of external osmolarity is the transmembrane protein kinase Sln1p that connect the cell membrane with the MAPK module by using a phospho-relay system (Ssk1p+Ypd1p) similar to the bacterial two-component system. Sln1p is a hybrid histidine kinase containing both a catalytic kinase domain and a receiver domain. The kinase is active when the osmolarity is low. In these conditions, a phosphate group is transferred from the His 576 residue in the catalytic domain of Sln1p to the Asp544 residue in the receiver domain of the response regulator Ssk1p, through several steps that include the participation of the intermediary protein Ypd1p (*1*). Phosphorylated Ssk1p seems to be the inactive form of this protein (*1*). Therefore, in high osmolarity conditions, the inactive Sln1p renders a unphosphrylated Ssk1p that is available to activate Ssk2p and Ssk22p (*56*). There is a second membrane osmosensor called Sho1p that is a transmembrane protein with a cytoplasmic SH3 domain. Sho1p transmits a signal to Pbs2p via the MAPKKK Ste11p, bypassing the need for Ssk2p and Ssk22p (*2*). Finally, a novel activation mechanism of Hog1p MAPK has been recently identified. This mechanism operates upon severe osmostress conditions (1.4 M NaCl) and is independent of the Sln1p and Sho1p osmosensors (*57*). Activation of the Hog1 pathway is transient. Two protein tyrosine phosphatases Ptp2p and Ptp3p are the major phosphatases responsible for the tyrosine dephosphorylation of Hog1p (*58*). Additionally, Hog1p regulates Ptp2p and/or Ptp3 activity at the posttranscriptional level, suggesting the existence of a negative feed back loop (*58*).

Under nonstress conditions, the MAPK Hog1p cycles between cytoplasmic and nuclear compartments. When activated by osmotic stress, the pathway induces the accumulation of Hog1p in the nucleus (*4,5*), from which is reexported after return to iso-osmotic enviroment or after adaptation to high osmolarity (*5*). The kinetics of increased nuclear localization correlated with the osmotic induction of a variety of genes, suggesting a role for Hog1p in gene expression, although the identification of the Hog1p kinase targets has remained elusive. Some, but not all, genes regulated through the HOG pathway contain a *cis* regulatory element known as the *stress response element* (STRE, discussed in detail above). It has been shown that STREs are specific targets of the HOG pathway (*59*). HOG pathway mutations almost abolish the STRE-mediated induction by osmotic stress, although the induction by heat-shock, nitrogen starvation and oxidative stress mediated by this regulatory element appears to be HOG-independent (*59*). However, Hog1p is not absolutely required for the osmotic induction of some STRE-containing genes such as *CYC7*, for which only a delayed induction is observed in the *hog1* mutant strain (*60*).

However, it is not clear if the only two identified STRE- binding factors, Msn2p and Msn4p, are targets of the HOG pathway, since HOG-mediated induction of STRE can still be observed in a *msn2msn4* double mutant, although at lower levels than in a wild type strain (*21*). Moreover, although osmotic stress induces nuclear accumulation of both Hog1p and Msn2/4p, the increase in nuclear concentration of these transcription factors can also be observed in a *hog1* mutant and vice versa (*3, 5*). So far, the only direct relationship between Hog1p and Msn2/4p has been established at the level of duration in the nuclear residence of Hog1p after osmotic stress (*5*). It has been suggested that Msn2/4p act by providing a nuclear anchorage for the kinase that might protect Hog1p against the action of specific protein phosphatases (*5*). Recently, two proteins involved in the osmotic activation have been identified: Hot1p and Msn1p. These proteins mediate the bulk of the Msn2p- and Msn4p-independent osmotic stress activation of HOG-dependent genes such as *GPD1*, *GPP2*, *CTT1* and *HSP12*. Finally, the Sko1p repressor, which binds to CRE-like sequence, mediates the osmostress signalling that, through the HOG pathway, regulates *ENA1* expression (*61*). However, whether these factors are direct targets of the HOG1 pathway has yet to be proved.

Genome-wide approaches have been used to analyse the transcriptional response to osmotic stress (*62, 63*). The results obtained indicate that this pathway is involved in the regulation of most of the osmotic induced genes. However, in many cases the induction was not completely abolished in the *hog1* mutant, suggesting the involvement of other signalling pathways (*62,63*). The list of genes whose expression is affected by the deletion of *HOG1* includes many of the genes that were affected by the deletion of *HOT1* or/and *MSN2/MSN4* (*62*). However, there are genes, such as *ALD2* and *ALD3* where transcriptional induction by osmotic stress depends on Msn2/4p but is independent on the HOG pathway (*64*).

In relation to the phenotypes of HOG pathway mutants, considering that the HOG pathway controls the osmotic induction of the glycerol producing enzymes *GPD1* and *GPP2*, and the role in yeast of glycerol as compatible solute in osmotic tolerance, it is not surprising that both *hog1* and *pbs2* mutants fail to grow in high osmolarity medium (*65*). However, *hog1* mutant is more sensitive to osmotic stress than the *gpd1* mutant (*66*), which reveals the importance of the HOG-dependent mechanism in the protection against osmotic stress. Moreover, recent observations suggest that the HOG pathway could have a physiological role in non-inducing conditions (*67*). Future work will clarify these aspects.

The PKC-pathway and the Cellular Integrity

S. cerevisiae contains a single homologue of mammalian protein kinase C coded by the *PKC1* gene (*68*). Pkc1p is part of a signaling pathway essential for growth whose main function is the maintenance of the cellular integrity (reviewed in *69–71*). The identification of additional components revealed that the core of the pathway consist in a MAPK cascade (Fig. 4). Pkc1p regulates the activity of the MAPKKK homologue Bck1p

(*72*), which controls the redundant pair of MAPKK Mkk1p and Mkk2p (*73*), which in turn regulate the MAPK Slt2p/Mpk1p (*74,75*). Three protein phosphatases have been shown to downregulate the pathway by dephosphorylating Slt2p. They are tyrosine phosphatases Ptp2p and Ptp3p (*76*), which also function on the mating and HOG MAPK pathways, and the dual specificity protein phosphatase Msg5p (*77*). Inactivation of any gene of the kinase cascade result in cell lysis due to defects in cell wall construction. It is worth to note that whereas *pkc1* mutants exhibit a nonconditional lytic phenotype, mutations in the components of the MAPK module result in cell lysis only at elevated temperature. The more severe phenotype of the *pkc1* mutants led to the proposal of a bifurcated pathway just after Pkc1p, with the MAPK cascade in one of the branch. At the moment the nature of this parallel pathway is still a matter of speculation.

Recently, upstream components of the pathway were characterized. A positive regulator of Pkc1p was identified as Rho1p (*78,79*). It belongs to a family of small GTP-binding proteins implicated in the organization of actin cytoskeleton (reviewed in *80,81*). Rho1p is localized at sites of active cell surface growth and plays an important role in bud formation, being required for both polarization of the actin cytoskeleton towards the bud and cell wall synthesis. Rho1p interacts with and regulates three more proteins besides Pkc1p, among them the 1,3-β-glucan synthase (*82,83*), the enzyme involved in the synthesis of the major structural component of the yeast cell wall.

Rho1p functions as a molecular switch between GTP-bound active and GDP-bound inactive forms. The switch is activated by two GDP/GTP exchange factor, Rom1p and Rom2p (*84*). Genetic studies suggest that a major role in signaling is played by Rom2p (*85,86*). The activity of Rom2p is regulated by the Tor2p (*85*), a protein that exhibits significant sequence homology to mammalian phosphatidylinositol kinases (see above), providing a possible link between phospholipid metabolism and cell wall synthesis and integrity. It has been proposed that a phosphorylated phosphoinositide might bind Rom2 and target its localization to the membrane, where it can activate Rho1p. On the other hand, inactivation of Rho1p is mediated by the GTPase activation proteins Bem2p and Sac7p and both have indeed been shown to down-regulate signaling to Slt2p (*77*).

If the PKC-pathway is going to respond to perturbation of cell surface (see below), the most upstream components in the pathway must be sensors associated with the membrane and/or the cell wall. Different approaches identified the transmembrane proteins Hcs77/Slg1/Wsc1 as a possible sensor for cell surface disturbances (*87–89*). Hcs77p belong to a small family of proteins that also includes Wsc2p, Wsc3p and Wsc4p (*89*). Mutants in *WSC* genes display phenotypes similar to the observed in mutants in the PKC-pathway. Genetic interaction places the function of Wsc proteins upstream of Pkc1p and Rho1p. More important, activation of Slt2p at high temperature (see below) depends on Wsc proteins. Hcs77p seems to contribute to a major extent that the others Wsc proteins to the regulation of the PKC-pathway. Recently, another putative sensor of cell wall

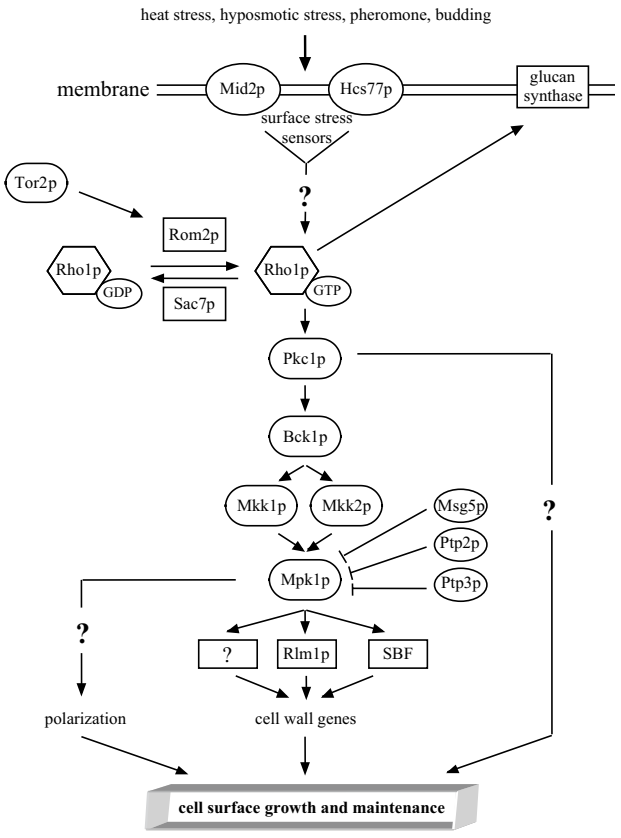

Fig. 4. The PKC-pathway

stress related to Wsc proteins was identified as Mid2p (*90*). Mid2p acts in parallel to Hcs77p in cell integrity signaling during vegetative growth, but its primary role is signaling wall stress during pheromone-induced morphogenesis. Hcs77p and Mid2p are highly glycosylated through long serine-threonine rich regions that span from plasma membrane to cell wall (*90,91*). This could be the domain that senses any stress applied to the exterior of the cell, leading to conformational changes in the protein which result in transduction of the signal to the inside. Future work will confirm or not this speculation.

The PKC-pathway is essential for the maintenance of cell integrity in response to environmental stresses and morphogenetic processes challenging cell wall strength. The PKC-pathway plays a physiological role in adapting to heat and hypoosmotic stress (*92,93*). Mutants in the MAPK cascade lysed at elevated temperature and an active pathway is also important for the induction of thermotolerance by mild heat shock. In fact, the pathway is activated by heat shock: a rise of the temperature induces increased levels of phosphorylated and activated MAPK Slt2p. It is thought that increased growth temperature causes physiological changes which create stress on the cell surface. Then, this stretching of the cell wall and/or membrane activates the PKC-pathway in order to maintain the cell integrity by stimulating cell wall biosynthesis. It has also been shown that the PKC-pathway is essential to survive an hypoosmotic stress. A shift to low osmolarity conditions induces an increase in the protein kinase activity of the MAPK Slt2p. Cell wall enables growing yeast to maintain an os-

motic gradient across their plasma membrane with higher osmolarity inside. A decrease in the external osmolarity will increase this osmotic gradient stretching the plasma membrane and the cell wall. This stress is likely again the physiological signal that leads the transient activation of the PKC-pathway, eventually inducing the cell-wall remodeling required for growth at the lower osmolarity.

The PKC-pathway is also activated during periods of polarized growth, like mating projection formation and budding (*94,95*). This activation is essential for the maintenance of the cell structural integrity in these morphogenetic processes. Wall stress due to the extensive remodeling and expansion of the cell surface during mating projection and bud formation probably provides the signal that activates the parthway in order to ensure cellular integrity.

The cellular responses triggered by the PKC-pathway in order to maintain cell integrity are begining to be characterized. The maintenance of the cell integrity occurs as the ressult of two combined events: the biosyntehsis of cell wall components and the assembly of a polarized actin cytoskeleton that directs secretory vesicles to the sites of surface growth. Different observations demonstrate that the PKC-pathway is activating both processes. Thus, mutations in many members of the pathway cause an aberrant distribution of the actin cytoskeleton and accumulation of secretory vesicles (*44, 96–98*). Besides, Slt2p mutant shows a synthetic lethal interaction with mutation in different components of the cytoskeleton (see *70*), and Rho1p, Mkk1p, Mkk2p and Slt2p have been localized at actin patches (*99,100*). These results suggest that the PKC-pathway regulates the establishment of a polaryzed cytoskeleton, although the molecular mechanism of this regulation is still unknown. On the other hand, the PKC-pathway induces biosynthesis of cell wall components by at least two mechanisms. First, Rho1p directly activates 1,3-β-glucan synthase at sites of surface growth (*82,83*). Second, it has been shown that many genes encoding proteins involved in cell wall biosynthesis are regulated by the PKC-MAPK cascade (*101–103*), indicating that, as is usual in MAPK cascades, the PKC-pathway trigger a transcriptional response. Recently, the MADS box transcription factor Rlm1p has been shown to be a downstream substrate of the PKC-pathway (*104,105*). Rlm1p interacts *in vitro* and *in vivo* with Slt2p. Moreover, it has been shown that Rlm1p transcriptional activity is regulated by direct phosphorylation by Slt2p and that Rlm1p mediates PKC-regulated gene expression. However, deletion of *RLM1* does not result in the cell lysis phenotype characteristic of mutants in components of the PKC-pathway and, at least for *FKS2* gene, regulation by the PKC-pathway is independent of Rlm1p (*102*). Because of this, it is assumed the existence of another yet unidentified target(s) mediating PKC-dependent regulation of expression of cell wall genes.

A second transcription factor related to the PKC-pathway is SBF. SBF is a heterodymer composed of Swi4p and Swi6p, which plays a crucial role in cell cycle progression at the G1/S transition (*106*). Genetic interactions and phenotypic similarities between SBF mutants and PKC-pathway mutants indicate that SBF is involved in the maintenance of cell integrity (*101,107*). In fact, many genes involved in cell wall biosynthesis are periodically expressed by SBF peaking at the G1/S boundary coincident with budding, when an active cell surface expansion and extensive wall remodeling occurs (*101*). Some of these genes are also regulated by the PKC-pathway (*101*), suggesting the possibility that SBF is a target of the PKC-pathway. Indeed, both Swi4p and Swi6p coimmunprecipitate with Slt2p and are phosphorylated *in vitro* by Slt2p, indicting that SBF is likely a direct target of Slt2p MAP kinase (*107*). However, because SBF and Pkc1p have different functions in the regulation of cell wall genes expression and, besides, other SBF-regulated genes are not under control of the PKC-pathway, the way in which regulation of SBF by the PKC-pathway affects its function is not well understood.

Besides its role in morphogenesis, several observations have claimed additional functions for the PKC-pathway such as DNA metabolism and in spindle pole body (SPB) duplication. It has been shown that PKC activity is required for *in vivo* phosphorylation of CTP synthetase and that Pkc1p can phosphorylate *in vitro* purified CTP synthetase, resulting in the activation of the enzyme (*108*). In addition, elevated rates of mitotic recombination are associated to a mutation of the *PKC1* gene (*109*). Different observations suggest that the DNA metabolism function of Pkc1p is independent from the cell integrity function mediated by the MAP kinase cascade (*110*). On the other hand, extensive synthetic growth defects were found when combining mutants from both PKC and SPB duplication pathways, and overexpression of multiple components of the PKC-pathway can suppress mutations in SPB duplication (*111*). Thus, the PKC-pathway certainly has more functions in yeast physiology than the well-known regulation of cell integrity, playing a more general role in the control of cell proliferation and growth.

Transcription Factors Directly Activated by Stress Conditions

The yeast heat shock factor

The heat shock transcription factors (HSFs) are a structurally and functionally conserved class of proteins that, in response to environmental stimuli, activate the transcription of genes (the *HSP* genes) encoding products required for the folding and unfolding of other proteins, targeting, assembly and disassembly of proteins in oligomeric structures, and the degradation of proteins that are improperly assembled or denatured.

In *S. cerevisiae*, there is a single *HSF* gene, *HSF1* (*112*). The structure of the yeast heat shock factor, Hsf1p, is similar to metazoan HSFs; it contains a helix-turn-helix DNA binding domain and coiled-coil hydrophobic repeat domain, which mediates its trimerization (*113, 114*). However, it differs from mammalians, fly and plant HSF's in the presence of two transactivation domains, which respond differentially to heat shock (*115, 116*). The amino-terminal activation domain (NTA) mediates a transient response to high temperatures, while the carboxyl-terminal activation domain (CTA) is requi-

red to regulate both a transient and a sustained response (*116*).

The *HSF* genes are constitutively transcribed and stored in a low-active form under normal conditions. In yeast, the requirement for Hsf1p at normal temperatures is concluded by the essentiality of the *HSF1* gene. Metazoan HSF are activated in response to heat shock by the conversion from monomer to homotrimer. Trimerization greatly increases the DNA-binding activity and the trimer binds to a short conserved DNA sequence, the heat shock element (HSE), located in the promoter of the *HSP* genes (reviewed in *112*). On the contrary, in *S. cerevisiae* and *Kluyveromyces lactis* HSF appears as a trimer at relatively high concentration before heat shock and they seem to be the only eukaryotic organisms in which HSF can be associated with HSE in the absence or presence of heat shock (*117,118*). However, although the *in vivo* DNA-binding activity of Hsf1p is enough to bind HSE prior to heat shock, temperature stress increases this DNA-binding activity (*119*). The increased binding after heat shock is observed to a greater extent in weak HSEs, showing deviations from the consensus HSE sequence (*119*). Post-translational modifications of HSF could also be involved in this activation. An increase of Hsf1p phosphorylation with temperature has been correlated with its transcriptional activation. (*116*). However, other observations suggest that hyperphosphorylation could serve to deactivate Hsf1p, allowing a transient response (*120*).

Several observations have led to the model of heat shock response autoregulation by heat shock proteins (*121*). The model proposes that heat shock would cause an increase in the concentration of missfolded proteins, the Hsp70 substrates, leading to a depletion of »free« Hsp70 and the activation of the HSF. In support of this model, it has been recently established that human Hsp70 associates *in vivo* and *in vitro* with HSF1 and this association represses HSF1 (*122*). As mentioned, yeast heat shock factor (Hsf1p) differs from metazoan HSFs in its constitutive binding to the HSE (*117,118*). However, this difference does not exclude a similar type of regulation by Hsp70, since human Hsp70 specifically interacts with the transactivation domain of HSF1 and negatively regulates the transcriptional activity without having effect on DNA binding (*122*). Besides, different genetic data point to the participation of the four cytosolic Hsp70, (encoded by the *SSA1-4* genes) in the regulation of heat shock response (*123*). Thus, in spite of its thermosensitivity, a *ssa1ssa2* double mutant strain survives a direct shift from 25 to 50 °C better than wild type (*124*). The increase in basal thermotolerance can be attributed to the constitutive expression in the *ssa1ssa2* mutant of other heat shock proteins, including Hsp104 which, in its turn, is indicative of the negative role of Hsp70 on heat shock gene expression (*124*). This effect occurs through the Hsf1p since mutating HSE can eliminate the constitutive expression of Hsp's (*125*). Further similar evidence has been obtained from a search for extragenic suppressors of the temperature sensitive phenotype conferred by the *ssa1ssa2* double mutation. One of the mutations, *EXA3-1*, is linked to the *HSF1* gene and causes a decrease in Hsf1p activity (*126*).

Characterisation of the phenotypes associated to mutations in specific Hsp's reveals that some of them are essential at any temperature or required for growth at temperatures beyond the normal range. Thus, cells carrying mutation in both the *HSC82* and *HSP82* genes are not viable, while a mutant in either of the two genes can grow only at temperatures below 37 °C (*127*). However, the hypothesis that Hsp's provide tolerance to heat and other stresses has frequently been the object of controversy (*128–130*). De Virgilio *et al.*, (*131*) reported that a mutant strain unable to synthesise proteins during a preconditioning heat treatment acquire thermotolerance, albeit to a lesser degree than the corresponding wild type strain. These results suggest that other factors, such as trehalose synthesis, can also play a role in stress tolerance. In relation to the general role of Hsp's in thermotolerance, one of the most intriguing reports was provided by Smith and Yaffe (*132*). These authors described a yeast strain containing a mutant allele of the heat shock factor, *hsf1-m3*, in which the acquisition of thermotolerance is not affected, although this mutant is defective in the induction of Hsp's. However, the finding that this mutant constitutively expresses high levels of Hsp104, a protein directly involved in thermotolerance (*133*), could be the reason for the strain's ability to acquire wild type levels of thermotolerance (*134*). Hsp104 contains two ATP-binding sites that are essential for its function in thermotolerance (*135*). Unlike other chaperones, Hsp104 functions have been shown as directly involved in rescuing heat inactivated proteins from insoluble aggregates (*136,137*). This function would be performed in conjunction with Hsp70 and Hsp40 chaperones. The product of the reaction catalysed by Hsp104, the dissaggregated protein, would be the substrate for the refolding activity of Hsp70 and Hsp40 (*137*). Recently, Hsp104 has also been found necessary for repairing heat-damage glycoproteins in the endoplasmic reticulum (*138*). Thus, yeast has developed a chaperone system that allows even aggregated proteins to be rescued. Those proteins that can not be repaired will be a target for degradation.

Yap1 and the response to oxidative stress

Yeast, as aerobically growing microorganism, has to manage with the generation of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical or superoxide anion. ROS are generated by normal metabolic processes (respiration or β-oxidation of fatty acids) as well as by exposure to pro-oxidants such H_2O_2 or heavy metals. They are potentially inducers of damage in lipids, proteins and nucleic acids (reviewed in *139, 140*). The adaptative response to ROS includes the induction of many genes, some of them with well-known antioxidant functions. Thus, using two-dimensional gel electrophoresis Gordon and coworkers (*141*) have identified 167 proteins which expression changes by treatment with H_2O_2. As expected, the list includes proteins with ROS scavenging activities and previously identified stress proteins, including Hsp's. The induced proteins would be required for the adaptative response to oxidative stress as the inhibition of this response by cycloheximide suggests (*140*). The analysis of the induced proteins also points out to metabolic enzymes as

H_2O_2-responsive targets, indicating metabolic fluxes redistribution in response to the oxidative agent. The changes in carbohydrate metabolism, which appear to be diverted to the regeneration of NADH, are remarkable. The possibility that different ROS induce different sets of genes has been investigated by comparing the pattern of proteins induced in response to H_2O_2 or to the superoxide generating agent menadione. It can be concluded that, although there are proteins specifically induced by H_2O_2 or menadione, a significant overlap between both responses exists (*142*).

Two transcription factors are especially important in the regulation of the adaptative response to oxidative stress: Yap1p and Skn7p, although only the regulation of Yap1p is known. It occurs through an increase in DNA-binding capacity, mainly through the control of nuclear localization. Under normal conditions Yap1p is restricted to the cytoplasm, but it becomes nuclear in response to oxidative stress (revised in *2*). Yap1p contains a C-terminal region with three conserved cysteine residues called the CRD (cysteine rich domain) and it has been proposed that the oxidation of these cysteines acts as a sensor of the redox state (*143*). The CRD is required for maintaining Yap1p in the cytoplasm, since it includes a nuclear export sequence (NES) through which the export factor Crm1p works to maintain Yap1 in the cytoplasm under non-stress conditions (*143,144*). The binding of Yap1p to Crm1p is inhibited in an oxidative environment, resulting in the nuclear localization of Yap1p (*144,145*). The current model suggests that under normal conditions Crm1p would continually export Yap1p from the nucleus. However, during oxidative stress, the oxidation of the cysteines contained in the CRD of Yap1p would lead to a reduction in the interaction between Yap1p and Crm1p, due to a reduced export and, as consequence, the accumulation of the transcription factor into the nucleus (*2*). Inside the nucleus Yap1p binds to the AP-1 response element (ARE: TGACTCA) that is located in the promoter of a number of yeast genes encoding antioxidants defences.

In relation to Skn7p, it contains a receiver motif homologous to that found in bacterial two components signal transduction systems (*146*) and a region similar to the DNA-binding domain of Hsf1p (*147,148*). This gene was also cloned as *POS9* in a screen for mutant hypersensitives to hydrogen peroxide (*149*), suggesting a role for Skn7p/Pos9p in the yeast oxidative response. Little is known about the activation of Skn7p by oxidative stress. Skn7p contains a conserved aspartate residue within the receptor domain that suggests its participation in the typical mechanism of transduction described for the two-components systems. However, phosphorylation of this residue is not necessary for the role of Skn7p in the oxidative response (*150*). Moreover, although it has been reported that the activity of Skn7p/Pos9p can be regulated through the sensor Sln1p/Ypd1 that controls the HOG MAPK, the role of Skn7p/Pos9p in the oxidative stress is independent of Sln1p and Ypd1p (*151*).

Both *yap*1 and *skn7* mutants show an increased sensitivity to H_2O_2 and tert-butyl hydroperoxide (t-BOOH), but only Yap1p is important for cadmium resistance (*152*). These phenotypes are likely to be the consequence of Yap1p and Skn7p requirement for the induction of many genes encoding defences against ROS. For some genes, such as *TRX2* and *TRR1*, both Yap1p and Skn7p are required for expression (*150*), but for others, they have opposite effects, with Yap1p acting positively and Skn7p acting negatively (*152*). The Yap1p and Skn7p-dependent group includes most of the known ROS scavenging activities whereas many of the genes encoding components of metabolic pathways regenerating reducing power are Skn7p-independent (*152*). Recently, it has been found that Skn7p is required for the full induction of the *HSP* genes in response to oxidative stress. Surprisingly, Skn7p can bind to the same regulatory sequences than the Hsf1p and interact *in vivo* with Hsf1p, suggesting a model whereby Skn7p becomes activated by oxidative stress and binds to the promoter of stress responsive genes through the formation of a Skn7p-Hsf1p complex (*153*).

Perspectives

It is clear that research during recent years has started to give satisfactory answers to how yeast cells face different stresses, but several major questions still remain unanswered. Diverse signalling pathways have been developed by the cells in order to first sense, and second respond to stress. Systems to adapt to specific stress, such as heat stress (HSF), oxidative stress (Yap1p) or osmotic stress (HOG pathway), as well as a general system induced by many stresses (the General Stress Response) have been studied extensively. Common to all of them is the regulation of specific transcription factors, which activates the synthesis of proteins required to survive stress. Control of nuclear localization of the transcription factor (Yap1p, Msn2/4p) or of a regulator of the transcription factor (Hog1p) is a recurrent mechanism in the different pathways. However, as yet we do not have a clear picture of the molecular mechanisms underlying stress sensing and pathway activation. This is probably the main gap in our knowledge of the path from the signal to the cell response in different stresses and one of the most important points to be solved.

More unanswered questions still exist about the PKA, PKC and TOR signalling pathways, whose involvement in stress response is indicative of their central role in cell physiology. What are the molecular bases of sensing cell surface or nutritional stress? How is this signal then transduced to activate the kinases of the pathways? What is the nature of the branched points in the pathways? Which other proteins are controlled by the pathways and how are they regulated? How do these pathways control other cellular processes?

Another important challenge will be to clearly determine how different pathways, activated under similar conditions of growth or stress, are coordinated to achieve an optimal response to stress. This coordination may involve cross-talk between pathways acting at the same time. Adaptation to nutrient conditions is a combination of the responses triggered by the Tor and PKA pathways, and a cross-talk is evident in this case as both pathways control subcellular localization of Msn2p and Msn4p transcription factors. The HOG and the PKC-pathway act under opposite conditions; do these path-

ways act independently of each other, or is there cross-talk at a high level? Another good example is the fact that different pathways are activated by heat shock: HSF, the general stress response, and the PKC pathway. It is convenient to look at the cell as a whole in order to know how different pathways work together in the response. Does coordination of the pathways involve sequential activation in order to carry out some processes before others? Or does coordination of pathways serve to modulate the strength and quality of the response depending on the intensity of the stress? Finally, it will be important to consider that many components of the signal pathways described above are lethal. It is necessary to learn more about the physiological functions of these pathways in non-inducing conditions. Future work will surely answer all the above questions. What we should expect then is a more integrated picture of the signalling pathways involved in controlling cellular function in response to stress.

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Signalizacija stresa u kvascu

Sažetak

Odgovor na uvjete okoliša obuhvaća složeni sustav puteva za prijenos signala. Oni omogućuju stanici da osjeti promjene u okolini, te prenese signal s površine stanice do jezgre. Tako se signali stresa pretvaraju u promjene ekspresije gena. Proteini sintetizirani pod tim uvjetima omogućuju popravak oštećenih stanica te ih zaštićuju od daljnjeg izlaganja stresu. U ovom su pregledu razmatrani različiti putevi prijenosa signala koji se aktiviraju pod uvjetima stresa u *Saccharomyces cerevisiae*. Opisan je napredak postignut posljednjih godina u istraživanju osjeta signala stresa, te kako se on prenosi u nukleus, zatim kako se aktiviraju transkripcijski faktori, te koji se geni induciraju.