Review

Stress Responses in *Saccharomyces cerevisiae*: The Role of PKA Signalling Pathway

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Abstract: The yeast *Saccharomyces cerevisiae* is widely used in food and nonfood industries. During industrial fermentations yeast strains are exposed to fluctuations in oxygen concentration, osmotic pressure, pH, ethanol concentration, nutrient availability and temperature. Fermentation performance depends on the ability of the yeast strains to adapt to these changes. Suboptimal conditions trigger responses to the external stimuli to allow homeostasis to be maintained. Stress-specific signaling pathways are activated to coordinate changes in transcription, translation, protein function, and metabolic fluxes while a transient arrest of growth and cell cycle progression occur. cAMP-PKA, HOG-MAPK and CWI signalling pathways are signal transduction pathways turned on during stress response. Comprehension of the mechanisms involved in the responses and in the adaptation to these stresses during fermentation is key to improving this industrial process. The scope of this review is to outline the advancement of knowledge about the cAMP-PKA signalling and the crosstalk of this pathway with the CWI and HOG-MAPK cascades in response to the environmental challenges heat and hyperosmotic stress.

Keywords: *Saccharomyces cerevisiae*; stress; cAMP-PKA; CWI; HOG-MAPK; crosstalk

1. INTRODUCTION

Microorganisms have evolved responses that allow them to survive stressful challenges in constantly fluctuating external environment. Cellular responses to external stress are rapid, highly dynamic, plastic, complex, and involve the coordinated stimulation of many different pathways for the regulation of the gene expression at different levels [1]. The readjustments allow equilibrate the effects of stress with the physiological requirements of the cell, guarantee that critical cell parameters are fine tuned to ensure cell survival.

The yeast *Saccharomyces cerevisiae*, a single-celled microorganism used to produce alcoholic drinks and bread, has also been widely used as genetic model system [2]. Yeast cells suffer the exposure to several types of stress as environmental conditions change, both in natural situations and during industrial processes. Both the damage caused by stress and the yeast response depend on the type and degree of stress and the developmental stage of the yeast at the time of the stimulus [3,4]. Regardless of the type of stress exerted on the cells, a general stress response is induced. Therefore, when yeast cells are exposed to a mild stress, an increased tolerance to other stresses is achieved and restoration of cellular homeostasis is facilitated [5,6]. When the buffering capacity fails to recover cellular homeostasis, cell death programs are stimulated to eliminate irreversibly damaged cells [7,8].

S. cerevisiae has evolved mechanisms to sense, respond and adapt to these environmental changes. These mechanisms include several signal transduction pathways. Yeasts are one of the pioneer organisms used to study in detail the feedback mechanisms, the

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structure, organization and cellular responses through several signalling pathways to different stresses. The signalling pathways, usually conformed by kinase cascades, allow a tight control of the response to a specific signal [9–11]. The compartmentalization of intracellular effectors, via adaptors or anchor proteins, is critical to the temporal and spatial control of signal transduction. Although several types of stress have been studied in yeast, the complete stress-activated network and the principles that control signal integration remain incomplete [12].

When *S. cerevisiae* grows in optimum environmental and nutrient conditions, expression of growth-related genes is high and expression genes involved in stress defense is low. One of the transduction pathaws involved in the regulation of this balance is that of cAMP-protein kinase A (PKA) [4,13,14]. Unfavourable conditions turn off this pathway and, at the same time, stress-specific signalling networks are activated and allow coordinated changes at the level of transcription, translation, post-translational modifications, and metabolic fluxes. This results in an appropriate response to each stress situation.

The well-known cAMP-PKA pathway responds to external stimuli through the modulation of the second messenger cAMP, which activates the PKA [15,16]. *S. cerevisiae* PKA is a tetrameric holoenzyme consisting of a regulatory subunit (Bcy1) dimer and two catalytic subunits (Tpk1, Tpk2 and Tpk3). A single gene *BCY1* encode the regulatory subunit, while there are three genes, *TPK1*, *TPK2* and *TPK3*, encoding the catalytic subunits [17]. When PKA is in its inactive state, the Bcy1 dimer is bound to two catalytic subunits (Tpk). In response to different stimuli, cAMP increases, and the Bcy1 dimer undergoes conformational changes that promotes the catalytic subunits release, which phosphorylate their target substrates [18–20]. The output is a wide variety of specific responses. The cAMP-PKA signalling pathway in *S. cerevisiae* has also been associated with the regulation of ageing, budding, actin repolarization, glycogen accumulation, stress resistance, sporulation, pseudohyphal differentiation, fermentative growth, stationary phase entry, and transcriptional regulation in response to different stimuli [21-25].

This article reviews the current state of knowledge of the cAMP-PKA pathway involvement and the crosstalk with the CWI and MAPK signallig pathways in the response to environmental challenges focusing in heat and hyperosmotic stress in *S. cerevisiae.*

2-. ROLE OF cAMP-PKA PATHWAY IN THE CELLULAR RESPONSE TO STRESS

Under stressful growth conditions, *S. cerevisiae* activates both transcriptional and physiological protective mechanisms. The stressed yeast cells activate specific transcription changes; thus, the expression of specialized genes is modulated to address the particular stress condition [26,27].

Genomic expression and global phosphoproteome studies shed light on the modulation of genes and protein phosphorylation involved in carbohydrate metabolism, protein folding degradation and processing [28-30]. The expression patterns of these genes during the adaptation to diverse stressful environments were termed as "Environmental Stress Response" (ESR) [3,31,32]. Actively growing cells are more sensitive to stress than quiescent cells [33].

In *S. cerevisiae*, one of the central controls of the ESR is the cAMP-PKA signalling, which transduces the changes in environmental conditions. The cAMP-PKA pathway is repressed in response to stress, and signalling pathways are activated to coordinate the transcriptional and translational modifications as well as the changes in the metabolic flux along with cell cycle arrest. The importance of this pathway in the adaptive response to stress is evident in mutants with hyperactive cAMP-PKA pathway. These mutants show very low tolerance to stress, decreased viability in stationary phase, and no trehalose and glycogen accumulation. On the other hand, mutations that decrease the PKA result in phenotypes with high tolerance to stress, increased accumulation of glycogen and trehalose, even in actively proliferating cells [34]. Furthermore, under many conditions, cAMP levels are high, resulting in the activation of PKA and accordingly the fermentative growth is promoted. However, in stationary phase, cAMP levels are low [35]. On the other hand,

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under stressful conditions, the cAMP levels decrease and, thus, the low PKA activity results in the inhibition of programs of genes that regulate growth and, at the same time, in the upregulation of stress responsive genes [14]

The promoters of most genes induced by the ESR contain the STRE element, the binding site for the non-redundant Msn2 and Msn4 transcription factors. Regulation by one or the other of these transcriptional factors depends on the promoter context and the type of stress. PKA regulates the nuclear localization and therefore the activity of Msn2/4. High PKA activity induces Msn2/4 phosphorylation, which maintains their cytoplasmic localization and thus suppresses their activity. On the contrary, when the PKA activity is low, Msn2 localization is predominantly nuclear and it is active [36–39]. The subcellular localization of Msn2 in yeast is dynamic occurring in bursts in response to rapid pulses of PKA activity [40–42]. Later results indicate that the phosphorylation of Msn2 by Tpk1 and Tpk3 isoforms leads to the inhibition of its activity, while Tpk2 seems to function as a partial activator of Msn2 [43]

2.1-. Osmotic Stress and PKA

An increased extracellular osmolarity generates hyperosmotic stress. The addition of high concentrations of salts as NaCl or KCl to *S. cerevisiae* cell cultures generates osmotic stress and ionic stress [44]. During the response to this stress, the concentration gradient promotes ion movement into the cell and the diffusion of water out of the cell to balance the osmotic pressure across the plasma membrane. The result is the sudden reduction in cellular volume and the cell cycle arrest. The cell responds rapidly by increasing the intracellular glycerol concentration, which causes water re-entering the cell. So, the original cell volume and turgor are restored [45].

S. cerevisiae responds to osmotic stress through two main mechanisms. One of them involves the osmolyte exporter Fps1. This channel remains closed upon hyperosmotic conditions preventing glycerol from exiting the cell [46,47]. The other mechanism involves the bona fide sensors, Sln1p and Sho1p, that control the HOG-MAPK (High Osmolarity Glycerol-Mitogen Activated Protein Kinase) pathway [48,49]. The MAPK of this pathway, Hog1, acts on cytoplasmic and nuclear targets to modify cellular metabolism to increase glycerol synthesis [50–52]. The HOG pathway includes a three sequentially acting protein kinases named MAPK, MAPK kinase (MAPKK, MAP2K), and MAPKK kinase (MAPKKK, MAP3K) [45,52]. There are two sensors that conform two signalling branches, SHO1 and SLN, which detect osmostress independently and activate MAP3Ks. Both Sln1 and Sho1 activate Pbs2 MAP2K by phosphorylation [53–56][57]. The activated Pbs2 can phosphorylate Hog1, and phosphorylated Hog1 translocates to the nucleus [58]. Hog1 allows the adaptive responses to osmostress of yeast cells, inducing the modulation of intracellular glycerol levels, metabolism, ion transporters, and translation. In addition, Hog1 regulates gene expression of osmostress-responsive genes [52,54,59]. The severity of the stress modulates Hog1 activation, which is negatively regulated by protein phosphatases [52].

The transcriptional regulation of Hog1 target genes occurs through diverse mechanisms, involving physical interaction with transcription factors as Msn2/4, Hot1, Tup1-Ssn6 and other transcriptional regulatory proteins [60,61]. Hog1 can also bind to the coding regions of stress-responsive genes and activates by phosphorylation the transcription elongation factors, Spt4 and Spt5 [62]. Recently, it has been proposed another mechanism by which Hog1 regulates the expression of genes by modulating the activity of the 5'-3' exoribonuclease Xrn1 [63]. Finally, it has been described the association of Hog1 to promoter regions of stress-responsive genes to facilitate the recruitment of RNA Pol II and the chromatin remodeling complexes SWI/SNF or INO80, allowing gene activation or repressing respectively [64–66].

PKA also regulates gene expression under osmotic stress in addition to doing so through the HOG pathway. It was demonstrated that PKA activity levels affect osmotolerance and modulate the expression of osmo-responsive genes in *S. cerevisiae* [67]. However, Hohmann et al proposed that PKA mediates ESR not only upon osmostress but also under several other stress conditions as high ethanol levels, thermal stress, oxidative

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stress, or nutrient starvation. Therefore, regulation by PKA is likely not specific to osmotic changes [68]. However, other results indicate that the regulation of ESR genes depends on the modulation of Msn2/4 activity by nuclear translocation [39,42,69,70], phosphorylation and degradation [38,71,72]. At some of these regulation levels, the signalling pathways cAMP/ PKA and HOG-MAPK have important roles [39,41,73–76].

In stress conditions, several protein kinases regulate gene expression through the binding to chromatin in either promoters or coding regions and through phosphorylation of histones, transcription factors, chromatin remodeler complexes and transcription machinery. PKA [77] and Hog1 [64,78] have been described as chromatin associated kinases. Baccarini et al demonstrated the importance of PKA chromatin association in the regulation of osmo-stress responsive genes. During osmotic stress Tpk1 accumulates in the nucleus, while Tpk2 and Bcy1 maintain the nuclear-cytoplasmic localization. The authors also demonstrated that in response to osmotic stress, PKA subunits bind to different gene regions of osmo-inducible genes. Both Tpk1 and Tpk2 subunits are recruited to the coding regions, and Tpk2 is also bound to the promoters of ribosomal protein genes. Tpk1 and Tpk2 mutant versions without catalytic activity do not bind the genes analysed so far. A mutant strain containing a deletion of *BCY1* gene which has a deregulated PKA activity, shows an increased Tpk1 but not Tpk2 recruitment. Furthermore, this mutant strain, shows a higher binding rate of the remodeler complexes SWI/SNF and INO80, and also, an upregulated gene expression under hyperosmotic conditions [79].

2.2-. Crosstalk between cAMP-PKA and HOG MAPK pathway during osmostress

On several occasions, the same stimulus can be processed by different signaling pathways in the cells. Likewise, there are diverse stimuli that lead to interaction and cross-activation between different signalling pathways. In this way, a specific response will be successful according to stress intensity, and modularity and hierarchical organization of the signalling pathways. The signalling pathways may interact through crosstalk or cooperative processes in response to a single stimulus [80]. In *S. cerevisiae* there are several examples in which multiple signalling pathways function in a coordinated manner to respond to stimuli.

The specific response to a signal of the different MAPK pathways described [70,81,82], which share several components, requires both insulation mechanisms and the coordinated communication among them. For instance, high osmolarity glycerol (HOG MAPK) pathways, mating programs (pMAPK) and filamentous growth (fgMAPK) can maintain the fidelity of the responses by restricting signalling complexes to discrete subcellular compartments and by switching on mechanisms to avoid crosstalk between MAPK cascades. In fact, in *hog1Δ* cells subjected to high osmolarity conditions, the pMAPK pathway is activated in contrast to wild-type cells. This way, the activation of the mating pathway is inhibited by Hog1 activity [83,84]. In response to osmotic stress, Hog1 also prevents the activation of the fgMAPK pathway by inhibiting the MAPKKK Ste11 of the SHO1 branch [52,84].

Among the MAPK pathways present in *S. cerevisiae*, the Cell Wall Integrity (CWI) pathway is key to overcoming cell wall damage caused by stressful conditions as chemical agents affecting cell wall biogenesis [85]. Several other stressors as heat stress, ethanol, hypo- and hyperosmotic shock, oxidative stress, among others that affect secondary the cell wall structure also activate CWI signalling [85,86]. A more detailed description of this signaling cascade is developed in the following section. Another example of crosstalk occurs during polarised growth in mating and in pseudohyphal development, where the activity of fMAPK and pMAPK pathways in coordination with the CWI to allow cell wall remodelling are required [87–89].

The relationship between the HOG and cAMP-PKA pathways in *S. cerevisiae* has also been described. The negative regulation of the HOG pathway mediated by PKA is required by yeast with defects in sphingolipid synthesis [90]. In addition, our own unpublished results indicate that the HOG MAPK and cAMP-PKA pathways interact during osmotic stress [91,92].

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PKA catalytic isoforms, Tpk1 and Tpk2, show different roles in the adaptive response to osmotic stress. The lack of *TPK2* gene improves the defective cell growth of *HOG1*-deficient strains under osmotic stress. Also, there is a negative correlation between *TPK2* expression and processes such as growth rate during the exponential phase, glucose consumption, and trehalose accumulation in osmotic stress condition in mutant yeast strains with a deletion in the *HOG1* gene. In contrast to *TPK2*, *TPK1* expression has a smaller effect on restoring the defective cellular response under osmotic stress in cells with an inactive HOG MAPK pathway. In addition, the phosphorylation of Hog1 induced by osmotic stress and Hog1 nuclear accumulation were unaffected by *TPK1* or *TPK2* deletions. Thus, the cAMP-PKA signalling is controlling the effectors that are downstream targets of the HOG-MAPK pathway [91] (Figure 1).

Pheromone stimulation initiates yeast mating, which triggers a MAPK cascade made up of Ste11, Ste7, and finally the MAPKs Fus3 and Kss1. During mating, cell cycle is arrested by high concentrations of pheromone,and polarized cell growth is induced to form cellular projections called “shmoos” morphology [93–95]. Yeast cells lacking *HOG1* gene show a "shmoo-like" morphology in response to osmotic stress due to the crosstalk between the HOG-MAPK and pMAPK pathways [84,96]. Our findings showed that the PKA catalytic subunits Tpk2 and, to a lesser extent, Tpk1, can reduce the crosstalk between the pheromone MAPK pathway and HOG-MAPK in a *hog1Δ* strain [91,92] (Figure 1).

In *S. cerevisiae,* filamentous growth is regulated by nutrient availability and the conserved filamentous MAPK the pathway (fgMAPK) [97]. However, the cAMP-PKA pathway activation is also required for this type of growth. Invasive growth is positively regulated by the cAMP-PKA pathway in response to glucose sensing and by the fgMAPK pathway in response to nitrogen-free medium [98,99]. Deletion of *TPK2*, but not *TPK1*, prevents filamentous growth. In addition, deletion of *TPK3* produces hyperfilamentous growth, indicating that Tpk3 is an inhibitor of this growth [99,100]. In hyperosmotic conditions, a *hog1Δ* strain exhibits invasive growth which is regulated by a crosstalk between the HOG1-MAPK and fgMAPK pathways [84].

The role of Tpk2 subunit on the crosstalk between the fgMAPK and HOG MAPK pathways was analyzed in a strain with deficiencies in the expression of *FLO8* gene. This strain is prevented from pseudohyphal growth [97]. In a *hog1Δ* mutant and under high osmolarity, the Tpk1 isoform is a positive regulator, but the Tpk2 isoform is a negative regulator of crosstalk between the fgMAPK and HOG MAPK pathways (Figure 1).

Hog1 and PKA can be chromatin-associated kinases (Pokholok et al., 2006). It was shown that Tpks and Bcy1 bind both coding regions and promoters of the osmoresponsive genes upon stress. The recruitment of Tpk1 and Tpk2 is completely prevented in yeast mutant strains carrying catalytic inactive versions [79]. The expression of *TPK2* and *HOG1* has a reciprocal impact on the binding kinetics of Tpk2 and Hog1 to the chromatin in response to osmotic stress. In addition, Tpk2 and Hog1 affect the association of Snf2 (SWI/SNF complex) and Mns2 to the promoters of osmostress responsive genes [91,92].

Overall, we suggest that when the cells fail to activate the HOG MAPK pathway, they might downregulate the cAMP-PKA pathway to produce a better adaptive response to osmostress. The lack of the *HOG1* gene leads to the inactivation of Tpk2, resulting in the insulation between MAPK pathways. Furthermore, this adaptive mechanism would involve changes in the dynamics of Tpk2 association to chromatin and, consequently, in the regulation of gene expression in response to osmotic stress [91,92] (Figure 1).

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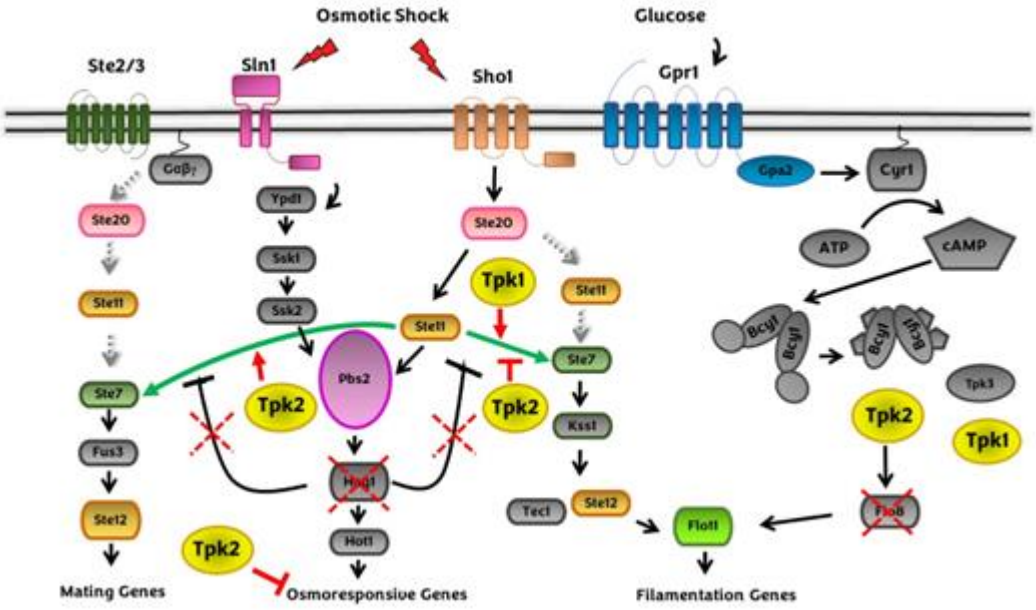

Figure 1. Model of the crosstalk between cAMP-PKA and HOG pathways in response to osmotic stress.

Two independent osmosensing mechanisms, the Sln1 and Sho1 branches, lead to the activation of specific kinases Ssk2/22 and Ste11 (MAPKKK) that converge on the common MAPKK Pbs2 which activates the Hog1 MAPK. *HOG1* deletion (red cross with dashed lines) promotes a reduction in cellular upon osmotic stress (NaCl). Deletion of *TPK2* restores the defective response of the *hog1Δ* strain. Hog1 inhibits the crosstalk with the fgMAPK pathway in response to osmotic stress by inhibiting MAPKKK Ste11 of the Sho branch. In a *hog1Δ* background, Tpk2 negatively regulates the crosstalk between the HOG-MAPK and fgMAPK pathways in response to osmostress. However, Tpk1 positively regulates the crosstalk between the HOG-MAPK and fgMAPK pathways in response to osmotic stress. In strains with the genetic background BY474, the transcription factor Flo8 is not expressed. In this strain, the invasive growth signalling by the cAMP-PKA pathway is impaired in the presence of glucose. The deletion of *HOG1* in the BY4741 strain promotes the crosstalk with the fgMAPK pathway upon osmotic stress. Tpk1 and Tpk2 catalytic subunits of PKA induce or inhibit the crosstalk between HOG and fMAPK pathways, respectively. Black arrows represent positive regulation, T symbols represent inhibition, the red cross with dashed lines indicates *HOG1* deletion, green arrows show activated pathways and grey dashed arrows represent off state.

2.3-. Thermal Stress and PKA

At suboptimal temperatures, different protective mechanisms are activated in *S. cerevisiae*, including a transcriptional gene expression program known as the Heat Shock Response (HSR) [27,101]. During this response the expression of genes involved in protein biosynthesis pathways are downregulated and heat-shock proteins genes are upregulated [102]. The HSR is also activated by other stresses such as heavy metals exposure, oxidative and alterations in protein conformation [103]. In addition, yeast cells modify the membrane composition and their metabolism [104]. The upregulation of heat-shock genes is driven by the transcription factor Hsf1 (Heat Shock Factor) [103]. This factor is inactive under non-stress conditions but active when misfolded proteins are accumulated in the cell. All these changes induced by thermal stress ensure the maintenance of proteostasis and metabolism in the cell [105]. In *S. cerevisiae,* the above mentioned Msn2/4 is a second kind of transcription factors that regulate the heat-shock gene expression. The expression regulation by Msn2/4 transcription factors is much extensive than transcripts induced by heat shock, since includes genes induced by other stresses in the general Environmental

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Stress Response. The shifts in the transcription levels of the HSR genes are the result of transcriptional changes and also differences in mRNA stability [106].

As it was mentioned before, PKA inhibits the Msn2/4 function, but in addition, other signal transduction pathways also regulate their activity in response to different environmental conditions, through factors as Mck1, Rim15, Yak1, Snf1, and Hog1 [43]. PKA activity is dispensable in the double deletion mutant strain of *MSN2* and *MSN4* genes. Therefore, the targets regulated by Msn2 and Msn4 stimulate genes that inhibit growth antagonizing the PKA dependent growth [36]. There are evidences that suggest that Yak1 kinase would fulfill this role [36,74].

In response to heat shock, Msn2/4, like Hsf1, is hyperphosphorylated; however, this modification is inhibited by cAMP. Therefore, the hyperphosphorylation might not be mediated by PKA [72]. However, heat shock slightly decreases cAMP levels through Ras activator, Cdc25, destabilization. Thus, cAMP/PKA could be the nexus between stimulus and response in HSR signalling although additional phosphorylation events may also act as regulating this response [107].

The assembling of the ribonucleoprotein (mRNP) composed of mRNAs and RNA-binding proteins (RBPs) is critical in the mRNA fate. During stress conditions, some mRNPs aggregate into larger complexes assembling membraneless organelles named RNP granules. There are many different types of cytoplasmic RNP granules; Stress Granules (SG) and Processing Bodies (PB) are two examples of them. Both types of granules participate in several aspects of mRNA metabolism as storage, localization, translation and decay [108–110].

However, how different types of stress impact in the formation of RNP granules is an unresolved question. PBs and SGs contain several groups of proteins as well as mRNAs, and these proteins participate in the biological activities definition of the granules. Among these proteins different protein kinases and phosphatases, have also been found associated to P-bodies [111–115]. PKA has a key role in the regulation of PBs and SGs assembling in response to glucose deprivation and stationary phase entry [114,116]. In addition, PKA regulates the assembly of PBs and SGs and protein translation upon heat stress in *S. cerevisiae*. It was shown that Tpk2, Tpk3 and Tpk1 isoforms have different roles in the assembling of SGs and PBs induced by thermal stress [117]. In conditions of moderate heat stress, Tpk3 aggregates and induces the assembly of proteins implicated in translation as eIF4G, Pab1 and eIF4E. However, these Tpk3 granules are neither PB nor SG. By contrast, upon severe heat stress the assembling of PBs and SGs containing both Tpk2 and Tpk3 and the 48S translation initiation complex are induced. The deletion of *TPK2* elicits a strong translational arrest and an increment in the number and size of SGs and PBs. On the contrary, the deletion of *TPK3* inhibits the assembling of SGs and PBs as well as the general protein translation.

Finally, *TPK1* has no effect on the SGs and PBs evoked by heat shock. The localization of Tpk2 is dependent on its kinase activity, but Tpk3 kinase activity is not necessary for its aggregation, indicating that each catalytic subunit isoform would play different roles in granule assembly in response to severe heat stress. Therefore, Tpk2 and Tpk3 have opposite roles on the general protein translation in response to heat stress showing that the same signalling pathway can generate different physiological responses [117].

2.4-. Crosstalk between cAMP-PKA and CWI pathways during heat stress

As mentioned before, high temperatures induce to the activation of the HSR and the CWI pathways in yeast cells. HSR response is regulated by the action of the transcription factors Hsf1 and Msn2/4. It was demonstrated that *TPK1* expression in response to heat shock depends on Msn2/4 but not on Hsf1 [118].

Heat-shock activates CWI pathway [119]. The first environmental condition related to the MAPK Slt2 activation was growth under thermal stress conditions [85,120]. Although the molecular mechanisms involved are not fully understood, changes in plasma membrane composition could participate to CWI activation by thermal stress [86]. There

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are different CWI sensors described, namely Wsc1–3, Mid2 and Mtl1 [121,122]. The participation of the CWI receptors in the sensing of this stimulus is not fully undestood. Yeast strains carrying single deletion mutations, *wsc1Δ*, *wsc2Δ*, or *wsc3Δ*, are thermo-tolerant, while the double mutants are thermosensitive [123]. The overexpression of Mid2 partially overcome the lack of *WSC1* [124]. Therefore, the sensors have overlapping functions although they are also specific. Subsequently, it was stablished that upon heat shock the Wsc receptors have an additive effect [125]. Downstream of the membrane sensors (Wsc1-3, Mid2 and Mtl), the signal is amplified by a MAPK cascade [121,122]. Through Rom1/Rom2 and the small G-protein Rho1, these sensors stimulate the downstream kinase Pkc1, which activates the MAPK cascade conformed by Bck1 and Mkk1/2. Finally, Mkk1/2 kinases activate the MAPK Slt2, and this kinase regulates the activity of Rlm1 and Swi4/6 transcription factors. The final result is the regulation of genes involved in cell wall biogenesis [85,102] (Figure 2).

CWI pathway is usually activated as a hierarchic top-down cascade; however, some stress stimuli can regulate this pathway at different steps of the cascade downstream Rho1. Some reports show that the activation of Slt2, the last kinase of the cascade, may come from another step in this MAPK cascade. Indeed, upon thermal stress, Slt2 is phosphorylated in a CWI sensor independent manner [126–128]. Thus, heat shock can activate the CWI signalling at the Mkk1/2 and/or Stl2 cascade steps [127] (Figure 2).

The crosstalk between the CWI and PKA signalling pathways was also studied. Yeast cells deficient in *IRA2* are not thermotolerant; however, the deletion of Wsc1 reverses this phenotype. The authors proposed that Wsc1 negatively regulates targets of RAS. Indeed, the deletion of Ras2 rescues the heat shock sensitivity of a *wsc1Δ* strain. Thus, Ras and Wsc1 have opposing effects on any downstream target [123]. Later, it was demonstrated that the Wsc1 sensor also contributes to the crosstalk between CWI with the cAMP-PKA pathway at the level of Slt2. It was described that Sdp1, a phosphatase that regulates negatively Slt2, is transcriptionally regulated by the transcription factors Msn2/Msn4 [129].

CWI signalling also plays a role in the regulation of *TPK1* expression during heat shock [130]. Previously, it was described that Tpk1 protein levels remain unchanged upon heat shock although *TPK1* mRNA is upregulated and the half-life of *TPK1* mRNA increases. This mRNA is localized in cytoplasmic foci that are not disassembled after cycloheximide treatment. The fact that these foci are resistant cycloheximide and results from the polysome profiling analysis indicate that *TPK1* mRNA is impaired for entry into translation. Therefore, in response to heat shock, Tpk1 levels are regulated by a post-transcriptional mechanism that involves the assembling of *TPK1* mRNA granules that are translationally silent. In this regulation the CWI components Wsc3 sensor and Mkk1 are necessary for *TPK1* expression upon heat-shock. However, the participation of Stl2 is not absolutely defined. The *TPK1* mRNA foci evoked upon thermal stress depends on Wsc3 but not on the other sensors. The levels of Tpk1 protein are lower in a *wsc3Δ* mutant than in a wild-type strain, and consequently PKA levels are also lower, as was demonstrated by phenotypes analysis. Regarding the participation of the transcription factors Swi4 and Swi6, it was published that apparently only Swi4 seems to be necessary for the regulation of *TPK1* expression [130]. It has been reported very little overlapping between the gene profiles of mutant strains *swi4Δ* and *slt2Δ* upon heat shock. Genes dependent on Swi4 but independent on both Swi6 and Slt2, such as *TPK1* [130], were described [127]. Therefore, the expression of Tpk1 subunit isoform in response to heat stress requires a crosstalk between CWI and cAMP-PKA signalling pathways (Figure 2).

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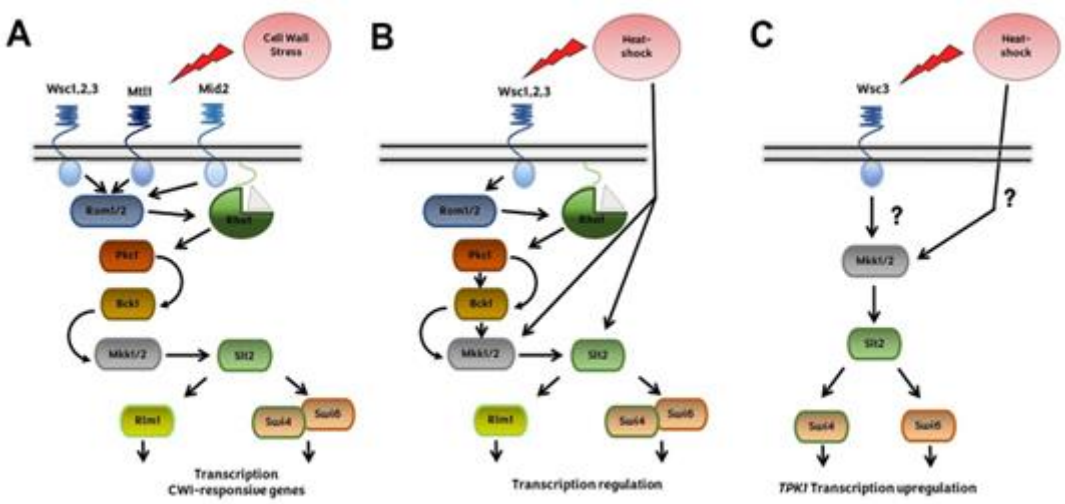

Figure 2. Crosstalk between cAMP-PKA and CWI pathway in response to heat stress.

A- Canonical CWI pathway. Damage in cell wall is sensed via wsc1-3, Mtl1 and Mid2 sensors that trigger the MAPK cascade conformed by Bck1, Mkk1/Mkk2, and Slt2 through Rho1 and the activation of Pkc1 the. **B**- CWI pathway and heat shock. The signalling pathway is activated by a different mechanism, regulating the kinases Mkk1,2 and Stl2 rather than Rho1 or Pkc1. Heat stress would be acting as a lateral input rather than operating in a linear "top-down" manner. **C-** Tpk1 expression regulation during heat shock. Tpk1 upregulation depends on the Wsc3 membrane sensor Mkk1 and the transcription factor Swi4.

3-. EFFECT OF STRESS ON THE SPECIFICITY REGULATION OF cAMP-PKA PATHWAY

Different external signals trigger the production of cAMP as the only second messenger in the cAMP-PKA signalling. Considering the multiple functions of this pathway in *S. cerevisiae*, an important question is how this kinase achieves specificity, that is, how the cell accomplishes the accurate substrate phosphorylation in response to different stimuli. The three Tpk isoforms are functionally redundant for cell viability despite each one performs specific functions [99,100,131–134].

The specificity of PKA signalling in *S. cerevisiae* is regulated by several mechanisms. We will describe these mechanisms highlighting those related to thermal and osmotic stress.

3.1-. PKA anchoring through Bcy1 interacting proteins

Yeast PKA localization appears to be different from that described for mammals. Bcy1 localization is variable and responsive to environmental and nutritional conditions [114,135]. Bcy1 N-terminus structure is similar to the canonical mammal RIIα domain (DD domain) as it has a helix-turn-helix motif and the critical amino acids for dimerization [136,137]. However, the binding domain of proteins described as Bcy1 interactors in *S. cerevisiae* displays different molecular features than the canonical domain of their mammalian counterparts, AKAPs (A-Kinase Anchoring Proteins, DD-AKAP), which contain essential hydrophobic residues [138–140].

The N-terminal domain of Bcy1 and two clusters of serine residues which are phosphorylated located at this domain have been reported to be critical for Bcy1 cytoplasmic localization in cells deprived of glucose [141]. Several Bcy1 interacting proteins have been described. Zds1, the first one, participates in the cytoplasmic localization of Bcy1 [141]. Other Bcy1-interacting proteins as Hsp60 (mitochondrial chaperonin), Eno2 (enolase II), and Ira2 (RAS GTPase-activating protein) were identified [142]. However, no interacting proteins were described in thermal or osmotic stress conditions.

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3.2-. Subcellular localization of Tpk1, Tpk2 and Tpk3 catalytic isoforms

In *S. cerevisiae*, the localization of each PKA subunit in different subcellular compartments and structures is affected by several environmental conditions, as glucose deprivation, thermal and osmostress or quiescent arrest [135,143]. When the yeast cells were grown in the presence of glucose, both Bcy1 and Tpk2 are localized in the nucleus; however, Tpk1 and Tpk3 subunits are equally distributed in nucleus and cytoplasm [114]. On the other hand, when yeast cells were grown in the presence of glycerol or when enter in stationary phase, both Tpks and Bcy1 subunits are localized mainly in cytoplasm [114].

As mentioned above, Tpk1 accumulates in the nucleus, whereas the localization of Tpk2 and Bcy1 does not change in response to osmostress [79]. On the other hand, upon heat stress, during glucose starvation or in quiescent cells, Tpk1 and Bcy1 display a diffuse cytoplasmic localization. However, Tpk2 and Tpk3 subunits are assembled in PBs and SGs [111,143,144]. The severity of the heat stress also regulates the localization of PKA subunits. In response to a mild heat stress, Tpk2 localization is cytoplasmic instead nuclear, and Tpk3 condensates in cytoplasmic foci which are different to classical SGs or PBs. On the other hand, both Tpk2 and Tpk3 subunits are assembled in SGs under severe heat stress [117]. When the cells are treated with cycloheximide and then subject to heat stress the foci containing Tpk2 and Tpk3 are not detected, indicating that these foci are dependent on the translation initiation repression [117]. The granular localization of Tpk2, but not that of Tpk3, depends on its catalytic activity. All these results suggest that different mechanisms are involved in the assembling of each catalytic subunit in response to severe heat stress [117]. A breakthrough in this topic is the demonstration that the N-terminus of Tpk2 subunit has a prion like domain necessary to localise this catalytic isoform to PBs and SGs upon heat stress, under glucose depletion and after quiescent arrest [145].

Therefore, there is a correlation between isoform specificity, subcellular localization and stress response. The differential subcellular localization of each catalytic isoform of PKA contribute to the specificity control of cAMP-PKA pathway as each isoform may interact with different proteins and potential substrates.

3.3-. Transcriptional regulation of PKA subunits

Pioneer high-throughput transcriptomic studies indicate that, in response to heat shock and saline stress, *TPK1*, *TPK2*, *TPK3* and *BCY1* gene expression is upregulated [26,146–150].

However, later published evidence demonstrated that the expression of each PKA subunit is differentially regulated under different growth conditions such as carbon source availability or growth phase [143,151]. In addition, PKA activity regulates the transcription of the three catalytic isoforms and Bcy1 subunits that compose the holoenzyme [118].

Tpk2 catalytic subunit is the isoform with the highest inhibitory effect on the activity of *TPK1* and *TPK3* promoters but fails to inhibit its own promoter [118]. Of all the subunits that compose PKA, only the expression of catalytic isoform Tpk1 is modulated during heat shock and osmostress. Under these conditions, both mRNA levels and half-life increase. In response to heat shock, the upregulation of *TPK1* depends on the transcription factors Msn2/4, Gis1, Sok2, and the kinase Rim15. During the *TPK1* promoter activation three positioned nucleosomes are evicted [118,152]. The chromatin remodeling involves the activity of the remodelers RSC and INO80 to maintain the repression of *TPK1* promoter under normal growth conditions, and the complex SWI/SNF to allow the activation after thermal stress [152]. Msn2/4 is necessary for the recruitment of the SWI/SNF complex. Strikingly, the catalytic subunits Tpk1 and Tpk2 are both recruited to the *TPK1* promoter upon heat shock but with opposite temporal patterns [152]. Furthermore, Tpk1 and Tpk2 catalytic activities have opposite effects on the chromatin remodeling of this promoter [152]. Therefore, a complex regulation mechanism involves the activity of Tpk subunits on the *TPK1* promoter. Finally, after thermal stress, the increased level of Tpk1 allows the conformation of PKA holoenzymes containing a higher proportion of the catalytic Tpk1

isoform. This holoenzyme might phosphorylate Tpk1 specific substrates improving the overall cellular fitness when normal environmental conditions are restored [130].

These results uncover a special mechanism involved in the regulation of the Tpk1 subunit expression by thermal stress that contributes to define the specificity of cAMP-PKA.

4-. CONCLUDING REMARKS

To respond adequately to stressors, *S. cerevisiae* employs different signalling pathways. Each pathway is fine-tuned through mechanisms that allow the specificity of the response. The complexity of the inputs to which the yeast may be exposed suggests that several pathways should be interconnected to process environmental signals and to achieve a specific response. Two important crosstalk interactions couple the signalling cAMP-PKA and CWI pathways in response to heat shock, and HOG-MAPK and cAMP-PKA pathways upon osmotic stress.

The regulation of the expression of each PKA subunit is one of the important mechanisms that allows signal transduction specificity. In response to heat shock and saline stress, *TPK1* is the only catalytic subunit of PKA upregulated, and the cAMP-PKA/CWI crosstalk coordinates Tpk1 expression.

The interaction of HOG-MAPK and cAMP-PKA pathways highlights the differential roles of the catalytic isoforms of PKA, Tpk1 and Tpk2, in the adaptive response to osmotic stress. The deletion of *TPK2* gene, but not *TPK1*, improves the defective cell growth of *HOG1* deficient strains under osmotic stress. PKA catalytic subunits Tpk2 and, to a lesser extent, Tpk1, can reduce the crosstalk between the pMAPK and the HOG-MAPK pathways in a deficient *HOG1* strain. The cAMP-PKA pathway activation is required for filamentous growth and each catalytic isoform has a different role in this process. The invasive growth of a *hog1Δ* strain under hyperosmotic conditions is regulated by a crosstalk between the HOG1-MAPK and fgMAPK pathways. Tpk1 is a positive regulator, while Tpk2 is a negative one in this crosstalk. Finally, there is also an interaction between PKA and *HOG1* at the level of transcriptional regulation of osmostress responsive genes. *TPK2* and *HOG1* have a reciprocal impact on the chromatin-binding kinetics of Tpk2 and Hog1. Also, both kinases regulate the binding of SWI/SNF complex and Mns2 to the promoters of osmostress-responsive genes.

In conclusion, intricated regulatory networks that include the crosstalk between different signalling pathways take place in response to stress. The complementation of signalling pathways, the fine tuning of the signals, and the specificity in the response to different stressors are key to produce a precise and timely gene expression output to overcome the stressful conditions.

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