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Multilayered control of gene expression by stress-activated protein kinases

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Stress-activated protein kinases (SAPKs) are key elements for intracellular signalling networks that serve to respond and adapt to extracellular changes. Exposure of yeast to high osmolarity results in the activation of p38-related SAPK, Hog1, which is essential for reprogramming the gene expression capacity of the cell by regulation of several steps of the transcription process. At initiation, active Hog1 not only directly phosphorylates several transcription factors to alter their activities, but also associates at stress-responsive promoters through such transcription factors. Once at the promoters, Hog1 serves as a platform to recruit general transcription factors, chromatin-modifying activities and RNA Pol II. In addition, the SAPK pathway has a role in elongation. At the stress-responsive ORFs, Hog1 recruits the RSC chromatin-remodelling complex to modify nucleosome organization. Several SAPKs from yeast to mammals have maintained some of the regulatory abilities of Hog1. Thus, elucidating the control of gene expression by the Hog1 SAPK should help to understand how eukaryotic cells implement a massive and rapid change on their transcriptional capacity in response to adverse conditions.

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Stress-activated protein kinases (SAPKs) are essential for cellular adaptation to extracellular stimuli

Exposure to stress requires rapid and efficient adaptive responses to maximize cell survival. Cells have sensing mechanisms and signal transduction systems designed to produce rapid outcomes in response to stress. There have been a number of signalling networks involved in stress

signal transduction and amongst them MAPK signalling network stands out. Eukaryotic organisms contain multiple MAPK families organized in discrete cascades. One of these is the SAPK cascade that has an essential role in proper cell adaptation to extracellular stimuli (Kyriakis and Avruch, 2001; Wagner and Nebreda, 2009). A prototype of the SAPK family is the yeast *Saccharomyces cerevisiae* Hog1 SAPK, the homologue of the mammalian p38 SAPK, which specifically responds to the increase in extracellular osmolarity and is required for cell adaptation to osmotic stress. There is a strong structural and functional preservation of MAPKs, as well as adaptive responses from yeast to mammals (Sheikh-Hamad and Gustin, 2004). Conservation of the stress MAPK cascades between yeast and human is indicated by the fact that individual kinases in the yeast pathway can be replaced by the corresponding human enzymes, that is, Hog1, Pbs2 and Ssk2 can be replaced by their mammalian counterparts p38 alpha, MKK3 and MTK1 respectively (Han *et al*, 1994; Moriguchi *et al*, 1996; Takekawa *et al*, 1997). Thus, new insights from yeast could be relevant to understand the role of SAPKs in mammals.

The central core of SAPK systems consists of a tier of kinases highly conserved among eukaryotic cells. Input to the central kinase module is usually achieved by different types of upstream sensors or activators. In budding yeast, the high-osmolarity glycerol (HOG) signalling system consists of two upstream independent branches that converge on the MAPKK Pbs2. The two branches are activated by completely different sensing mechanisms (Figure 1). One mechanism involves a two-component osmosensor (Sln1–Ypd1–Ssk1), similar to those on bacteria and plants, which appears to be sensing membrane turgor. The two-component system directly regulates the activity of the Ssk2 and Ssk22 MAPKKs. The second sensing system is extremely complex and is not completely characterized. It involves the mucin-like proteins (Hkr1 and Msb2), which likely act as osmosensors that activate Pbs2 in collaboration with a number of proteins, that include the transmembrane protein Sho1, Opy2, the small G-protein Cdc42 and the PAK (p21-activated kinase) family kinase Ste20, Ste50 and the Ste11 MAPKKK (Chen and Thorner, 2007; Hohmann *et al*, 2007; Tatebayashi *et al*, 2007; de Nadal *et al*, 2007). Once Pbs2 gets activated, it phosphorylates Hog1. In response to osmotic stress, phosphorylation and nuclear localization of the SAPK occurs in seconds and, depending on the strength of the osmotic stress, may last from 10 to 100 min (Hohmann *et al*, 2007). Therefore, activation of the Hog1 SAPK is extremely rapid but transient, suggesting that strong downregulatory mechanisms must exist to prevent extended Hog1 activation. Downregulation of signalling is absolutely required, as sustained activation of Hog1 is detrimental to cell growth (Maeda *et al*, 1994).

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Figure 1 Hog1 stress-activated protein kinase (SAPK) signalling. Two independent upstream osmosensing mechanisms lead to the activation of specific MAPKKs. A 'two-component' osmosensor (Sln1-Ypd1 and Ssk1 proteins) regulates the Ssk2 and Ssk22 MAPKKs, whereas the second branch of the pathway activates the Ste11 MAPKKK through the transmembrane protein Sho1 and the mucin-like proteins Msb2 and Hkr1. In response to osmotic stress, Pbs2 integrates both signals and activates the Hog1 SAPK, which induces a set of osmoadaptive responses.

A major mechanism for downregulation is related to intracellular osmolyte concentration. Adaptation to osmotic stress requires the accumulation of intracellular osmolytes such as glycerol or ions to increase the total intracellular solute concentration, thereby providing osmotic stabilization. Several evidences indicate that it is actually the ability of the sensors to detect an osmotic imbalance between the inside and outside of the cell rather than specific extracellular osmolarity that determines the duration of Hog1 phosphorylation. Basically, the sensors limit the input signal to the signalling system once the cell has started the process of adaptation. Therefore, production and transport of glycerol are key determinants of the signalling process (e.g., regulation of Fps1 glycerol channel, glycolytic flux and glycerol production) (Klipp *et al.*, 2005; Hohmann *et al.*, 2007). Regulation of SAPK signalling pathways has been associated with protein dephosphorylation. Indeed, dephosphorylation of kinases in the HOG pathway is accomplished by protein tyrosine phosphatases (e.g., Ptp2 and Ptp3) and Ser-Thr protein phosphatases of the PP2C family (e.g., Ptc1). Some of them are even regulated in response to osmotic stress. The role of the phosphatases is important to constantly counteract pathway activation and to adjust the dynamic range of response rather than the deactivation of the pathway during adaptation (Klipp *et al.*, 2005; Hohmann *et al.*, 2007). Several feedback regulatory loops exist in the HOG pathway, where in the same Hog1 SAPK targets upstream components of the system (e.g., Sho1, Ste50) (Hao *et al.*, 2007, 2008), although targeting of elements on the Sln1 branch of the pathway by SAPK appears to be the most critical for the dynamics of

signalling (Mettetal *et al.*, 2008; Macia *et al.*, 2009; Muzzey *et al.*, 2009). The feedback loops are predicted to be important to alter the dynamics and specificity of the system. Typically, systems with negative feedback show higher robustness against external and stochastic noise (Becskei and Serrano, 2000; Gardner and Collins, 2000), thereby increasing the efficiency in signal transmission. However, the presence of negative feedbacks introduces several constraints on the dynamics of the systems, because they impose delays in the response and reduce the sensitivity. The HOG pathway has counteracted responsiveness caused by feedback loops by intrinsic high-basal signalling even in the absence of stress. This allows for a fast-responsive system, which is able to detect small variations in extracellular osmolarity (Macia *et al.*, 2009).

The HOG signal transduction network is necessary for adaptation to osmotic stress. However, osmotic stress not only induces osmolyte accumulation, but it also has a deep impact on different aspects of cellular physiology. Control of cell-cycle progression has been shown to be very important for cellular adaptation to stress. The Hog1 MAPK modulates a rapid and transient delay of the G₁, S and G₂ phases of the cell cycle to permit the full development of adaptive responses before cell-cycle progression (Clotet and Posas, 2007; Yaakov *et al.*, 2009). In addition, stress induces cytoskeleton reorganization, changes in cell-wall dynamics and ion homeostasis and metabolic adjustments, as well as profound effects on gene expression (Hohmann, 2002; Hohmann *et al.*, 2007).

SAPKs have a key role in the regulation of stress-mediated gene expression

The role of gene expression in osmoadaptation is still not completely understood. It is clear that long-term adaptation to high osmolarity requires transcription and there are a known number of mutants of the transcriptional machinery that render cells osmosensitive (de Nadal *et al.*, 2004; Zapater *et al.*, 2007; Mas *et al.*, 2009). However, a recent report shows that a Hog1 that has been tethered at the membrane abolishes transcription at low osmolarity and is still competent to suppress the osmosensitivity of a *hog1* strain. Interestingly, it appears as the control of glycerol production might be sufficient for the maintenance of osmotic balance under those experimental conditions (Westfall *et al.*, 2008). Nevertheless, modulation of gene expression in response to osmotic stress reflects the rapid and transient response of Hog1 activation (Lopez-Maury *et al.*, 2008). Therefore, the analysis of the transcriptional output in response to stress by the Hog1 SAPK is an interesting model to understand how cells adjust a full transcriptional programme that is fast, precise and extremely efficiently in response to the extracellular stimuli.

Global transcriptional responses to stress have been studied in detail using gene expression profiling in both *Saccharomyces cerevisiae* and *Saccharomyces pombe*. In response to osmotic stress, there are a large number of genes whose transcription is induced and among them there is a set of specialized genes specifically required for osmotic stress, whereas another set of genes respond indiscriminately to different type of stresses. This last group of genes is known as the environmental stress response (ESR) in budding yeast or the core ESR (CESR) in fission yeast. The environmental stress response consists of about ~300 to ~600 genes whose

expression is induced or repressed by stresses, such as DNA damage, heat shock, osmotic stress or oxidative stress among others (Gasch *et al.*, 2000; Causton *et al.*, 2001; Chen *et al.*, 2003; Capaldi *et al.*, 2008). The extent and kinetics of the ESR or CESR appear to be dependant on the severity of the stress, as cells exposed to increasing stress often display broader changes in gene expression. It is also remarkable that this general stress response has been related to cross-protection, wherein exposure to a non-lethal dose of one stress can protect against potentially lethal doses of seemingly unrelated stresses (Berry and Gasch, 2008). Both the ESR and CESR, include genes involved in carbohydrate metabolism, defence against reactive oxygen species, protein metabolism, intracellular signalling and DNA damage. On the other hand, most of the genes repressed are involved in protein synthesis and in growth-related processes (reviewed in Gasch, 2007).

It has been clearly established that SAPKs have a key role in the regulation of transcription upon stress. In *S. pombe*, the SAPK Sty1 is activated by several stresses, including heat shock, oxidative stress, osmotic stress, starvation and DNA damage (Degols *et al.*, 1996; Degols and Russell, 1997; Toone *et al.*, 1998; Chen *et al.*, 2008), whereas in *S. cerevisiae*, the HOG pathway is mainly responsible for osmotic stress (de Nadal *et al.*, 2007). Nearly all genes induced or repressed in the CESR require Sty1 for proper modulation (Chen *et al.*, 2003). Correspondingly, in *S. cerevisiae*, where the ESR is not governed by one regulatory system but by different signalling pathways and transcription factors, the Hog1 SAPK is critical for regulation of ESR genes upon osmotic stress (Posas *et al.*, 2000). In addition to CESR/ESR, other genes that are not shared among different stresses have specific roles in adaptation to particular stresses and are also, in different degrees, under the control of SAPKs. In fact, a more accurate view of the effect of Hog1 in gene expression comes from studies using a *hog1* mutant strain. Those studies, averaging different stress conditions and different times upon stress, indicated that of all the genes regulated upon osmotic stress approximately one-third of them completely depend on Hog1 to be transcribed, one-third show different degree of dependence of the SAPK and one-third is independent on Hog1. Thus, this clearly indicates a major role for the SAPK in controlling gene expression (Posas *et al.*, 2000; O'Rourke and Herskowitz, 2004). The analysis of the complete transcriptional program mediated by Hog1 in *S. cerevisiae*, together with the binding of different transcription factors, showed that, in response to osmotic stress, SAPKs can specifically regulate and integrate at different promoters the stress response. This is accomplished basically by modulation of the individual contribution of transcription factors such as Msn2/Msn4, Sko1 and Hot1 in a promoter-specific context. This yields a complex and highly specific transcriptional network controlled by Hog1 (Capaldi *et al.*, 2008; Ni *et al.*, 2009).

SAPKs regulate transcription initiation by several mechanisms

The transcription process includes the assembly of the pre-initiation complex (PIC), which involves the recruitment of RNA Pol II and general transcription factors to DNA, initiation, elongation and termination. Studies from yeast to human have shown that SAPKs regulate eukaryotic gene expression in response to extracellular stimuli through

different mechanisms in different stages of the transcription cycle. However, a uniform view of the impact of SAPKs in transcription has not yet been defined. In yeast, studies on the role of Hog1 in transcription regulation have illustrated that SAPKs have not only one but multiple regulatory roles along the transcription process. These results, summarized here, challenge the classical view of transcription regulation by signalling kinases.

Hog1 directly phosphorylates and regulates the activity of transcription factors

As mentioned before, osmotic stress induces a massive loss of water from inside the cell. To overcome a dramatic increase in intracellular osmolarity, cells need to regulate the ionic balance before they are competent for transcriptional induction. This is especially dramatic in the presence of very high osmolarity. The Hog1 SAPK is critical to re-establish this balance and permit the assembly of components of the transcriptional machinery onto DNA as a pre-requisite towards transcription competence (Proft and Struhl, 2004).

The best-characterized function of SAPKs is their role in the regulation of gene expression at initiation (Figure 2). The Hog1 SAPK regulates several unrelated transcription factors, each of them responsible for controlling expression of a subset of osmo-responsive genes directly or in collaboration with other factors (Capaldi *et al.*, 2008; Ni *et al.*, 2009). These are the Hot1, Smp1, Msn1, Msn2 and Msn4 activators and the Sko1 repressor (de Nadal and Posas, 2008) that can act independently or in combination at specific promoters to elaborate a dynamic transcriptional response to stress (Ni *et al.*, 2009). The best understood mechanism by which SAPK modulates initiation of transcription in response to stress is the direct phosphorylation of promoter-specific transcription factors. Examples of this mechanism are reported in yeast with the MEF2-like activator Smp1 (Yu *et al.*, 1992) and the ATF/CREB-family member Sko1 (Nehlin *et al.*, 1992; Vincent and Struhl, 1992). *In vivo* co-precipitation and phosphorylation studies showed that Smp1 and Sko1 interact with Hog1

Figure 2 Stress-activated protein kinases (SAPKs) regulate transcription initiation of stress genes through several mechanisms. SAPKs may modulate initiation of transcription in response to stress by (1) direct phosphorylation of promoter-specific transcription factors; (2) stimulation of the recruitment of RNA Pol II and coactivators to the promoters; and (3) recruitment of the Rpd3 histone deacetylase complex and other chromatin modifying activities.

and they are directly phosphorylated upon osmostress in a Hog1-dependent manner. Phosphorylation of these transcription factors by Hog1 is important for its function, as mutant alleles unable to be phosphorylated display impaired stress gene expression (Proft *et al*, 2001; de Nadal *et al*, 2003). Sko1 has an extra layer of complexity as it acts as a transcriptional repressor in the absence of stress. Sko1 represses stress-induced gene expression by recruiting the general co-repressor complex Ssn6 (Cyc8)-Tup1 to target promoters (Garcia-Gimeno and Struhl, 2000; Pascual-Ahuir *et al*, 2001). Induction of Sko1-dependent genes requires the release of repression and this process is completely dependant on Hog1 (Pascual-Ahuir *et al*, 2001; Proft and Struhl, 2002). In fact, Hog1-dependent phosphorylation switches Sko1 from being a repressor into an activator modifying its association with Tup1-Ssn6 and allowing the recruitment of the chromatin remodelling complexes SAGA and SWI/SNF to osmostress inducible promoters (Proft and Struhl, 2002; Guha *et al*, 2007; Kobayashi *et al*, 2008). In mammals, numerous transcription factors are phosphorylated and regulated by p38 SAPKs, including MEF2, ATF2, ELK1, p53, MRF4 and CHOP among others (reviewed in Perdiguero and Munoz-Canoves, 2008).

Hog1 associates with DNA at stress-responsive promoters via specific transcription factors

The effect of Hog1 on transcription factors is not limited to their direct phosphorylation. For instance, the transcriptional regulators Msn2 and Msn4 control induction of expression of genes through the so-called stress response element in response to several stressful conditions, including osmostress (Martinez-Pastor *et al*, 1996; Schmitt and McEntee, 1996). Global expression analyses have uncovered a remarkable correlation between *MSN2/MSN4* and *HOG1*-dependent gene expression (Rep *et al*, 2000; Capaldi *et al*, 2008), although it is not clear how Hog1 regulates the activity of Msn2. Similarly, Hog1 interacts with Hot1 (Rep *et al*, 1999), a transcription factor that affects the expression of a small subset of Hog1-dependent genes, including *GPD1* and *GPP2*, involved in glycerol biosynthesis, and *STL1*, which encodes a glycerol proton symporter (Rep *et al*, 2000; Ferreira *et al*, 2005). It was also shown that the nuclear retention of Hog1 upon stress was dependant on the presence of these transcription factors, thus suggesting that they could act as nuclear anchors for SAPKs by engaging in stable interactions with them (Reiser *et al*, 1999; Rep *et al*, 1999). Strikingly, the phosphorylation of Hot1, and possibly Msn2, by Hog1 is almost irrelevant in stress-induced gene expression (Alepuz *et al*, 2003). A remarkable discovery on how Hog1 modulates gene expression is that in response to stress the kinase cross-links with several target promoters and this binding is mediated through physical interaction with specific transcription factors that recruit the SAPK to chromatin. For instance, recruitment of Hog1 to the *STL1* promoter depends on the activator Hot1, whereas recruitment of the kinase to the *CTT1* promoter depends on the transcription factors Msn2 and Msn4 (Alepuz *et al*, 2001). It is worth noting that the binding of Hog1 is only restricted to osmoresponsive genes (Pascual-Ahuir *et al*, 2006; Pokholok *et al*, 2006; Proft *et al*, 2006). The accumulation of Hog1 into the nucleus is not sufficient for its association with chromatin because the addition of a nuclear localization signal on the SAPK does not result in enhanced

chromatin association. However, binding to chromatin depends on its catalytic activity (Alepuz *et al*, 2001). Thus, although in a more traditional scenario, MAPKs would control the activity of a transcriptional regulator, the presence of Hog1 at target promoters already indicates that Hog1 itself might be having an important role on the regulation of transcription initiation (Alepuz *et al*, 2001; Chellappan, 2001; Proft and Struhl, 2002). It is worth noting that other MAP kinases from yeast, such as Fus3, Kss1 and Mpk1 are also recruited to chromatin (Pokholok *et al*, 2006; Kim *et al*, 2008). Similarly, structurally and functionally unrelated signalling kinases, including Snf1 (Lo *et al*, 2005), Tor1 (Li *et al*, 2006) or PKA (Pokholok *et al*, 2006), have been described in yeast to be recruited to chromatin.

This scenario is not restricted to budding yeast. In *S. pombe*, activated Sty1 SAPK phosphorylates a number of targets including the bZIP transcription factor Atf1, the fission yeast homologue of mammalian ATF2 (Shiozaki and Russell, 1996; Wilkinson *et al*, 1996). Atf1 binds to its cognate site as a dimer with a second bZIP protein, Pcr1 (Watanabe and Yamamoto, 1996). However, Atf1 phosphorylation by Sty1 is not essential for stress-induced activation of Atf1 target genes, but rather serves to positively regulate the stability of the Atf1 protein (Lawrence *et al*, 2007). As Hog1, activated Sty1 is recruited to Atf1-dependent genes in response to various stresses by association with Atf1 and its binding partner Pcr1 (Reiter *et al*, 2008).

Given the evidence from yeast and the conservation of SAPK functions in higher eukaryotes, mammalian p38 might well be an integral part of the transcriptional machinery. Indeed, during skeletal myogenesis, the p38 SAPK is recruited to chromatin and targets the SWI-SNF chromatin-remodelling complex to muscle-regulatory elements possibly via MyoD and/or its partner E47 (Simone *et al*, 2004; Lluís *et al*, 2005; Serra *et al*, 2007). Furthermore, phosphorylation of MEF2D by p38 is required for TrxG recruitment to myogenic loci (Rampalli *et al*, 2007). Similarly, the ERK1 MAPK and its target MSK1 are also recruited to genes in response to progesterone (Vicent *et al*, 2006; Crump *et al*, 2008). It is interesting to note that binding of ERK1 to the FOS promoter is mediated by the ELK1 transcription factor (Zhang *et al*, 2008). Taken together, the data suggest a new and widespread role for signalling kinases on chromatin regulation (Chow and Davis, 2006; Edmunds and Mahadevan, 2006).

Hog1 mediates assembly of the PIC at stress-responsive promoters

The observation that Hog1 kinase activity is needed for transcriptional activation, albeit phosphorylation of transcription factors is not an absolute requirement for transcription initiation, indicates that Hog1 can induce activation of gene expression by a mechanism other than the phosphorylation of the activator. Recruitment of the RNA Pol II machinery to osmoresponsive genes depends on both active Hog1 and the presence of specific transcription factors. Furthermore, the tight association of Hog1 with the largest subunit of RNA Pol II and the fact that the artificial tethering of the SAPK to DNA is sufficient to induce gene expression upon stress suggest that Hog1 might serve to recruit the basic transcriptional machinery to stress-responsive promoters (Alepuz *et al*, 2003).

Genetic and biochemical data suggests that in addition to binding transcriptional activators and facilitating RNA Pol II recruitment, SAPKs are important to recruit at promoters basic transcription complexes such as SAGA, Mediator and SWI/SNF. Several observations indicate that whereas Mediator is crucial for proper gene induction upon both mild and high osmotic stress conditions, the role of SAGA is dependant on the strength of the osmotic stress. Thus, the requirement for a given transcriptional complex to regulate a promoter might be determined by the strength of the stimuli perceived by the cell through the regulation of interactions between transcriptional complexes (Zapater *et al*, 2007). The recruitment of SWI/SNF also depends on the presence of Hog1 and, although elimination of components of these complex do not lead to clear effects on transcription, it might indicate that modification of chromatin could be important for efficient transcription in response to osmotic stress (Proft and Struhl, 2002).

The Rpd3 histone deacetylase complex is important by Hog1-mediated gene expression

Although histone deacetylation has classically been associated with the repression of gene expression (Robyr *et al*, 2002), there are many examples of particular genes in which a decrease in histone acetylation is associated with transcription induction (reviewed in Bernstein *et al*, 2000; Shahbazian and Grunstein, 2007). In addition to osmotic stress, Rpd3 deacetylase regulates the transcriptional activation of DNA damage-inducible genes (Sharma *et al*, 2007) as well as of the anaerobic *DAN/TIR* genes (Sertil *et al*, 2007), or oxidative and heat stress responses (Alejandro-Osorio *et al*, 2009). It is worth noting that studies in higher eukaryotic cells reported that the mSin3A-HDAC complex is required for embryonic stem cell proliferation because of its role in positively regulating the expression of Nanog (Baltus *et al*, 2009).

A genetic screen designed to identify mutations that render cells osmosensitive at high osmolarity showed that in addition to SAGA and Mediator, the Rpd3 histone deacetylase complex also has an important role in osmotic stress gene expression. Rpd3 is a member of a family of five related histone deacetylases in yeast and it has been described to regulate expression of a large number of genes (Yang and Seto, 2008). There are two known Rpd3 complexes that share a core of three subunits, including Rpd3, Sin3 and Ume1. Although the large Rpd3L complex is recruited to promoters and functions in transcription initiation, the small Rpd3S complex is involved in suppressing spurious intragenic transcription during elongation and in controlling promoter fidelity (Carrozza *et al*, 2005; Keogh *et al*, 2005; Li *et al*, 2007b,c; Biswas *et al*, 2008). Cells deficient in Rpd3 and other components of the large Rpd3L complex are osmosensitive and showed compromised expression of osmotic stress-responsive genes controlled by Hog1. Hog1 binds and recruits the Rpd3 complex to stress-specific promoters which leads to a reduced entry of RNA Pol II and deficient gene expression (de Nadal *et al*, 2004). It should be mentioned that the role of Rpd3 in osmotic stress promoters might not be restricted to altering chromatin structure, but might also provide a unique binding surface or recognition motifs for the recruitment of activators, as it has been proposed for acetylation and deacetylation in gene expression (Millar and Grunstein, 2006).

Hog1 regulates elongation in stress-responsive genes

Elongation is also a critical phase of transcription susceptible to strong regulation and the modification of the RNA Pol II carboxy-terminal domain (CTD) is just an example of it (Saunders *et al*, 2006; Egloff and Murphy, 2008; Fuda *et al*, 2009). Dynamic phosphorylation of Ser2 and Ser5 or even Ser7 of the CTD tandem repeat provides a platform for the recruitment of the appropriate factors at different phases of the transcription cycle (Chapman *et al*, 2007; Egloff *et al*, 2007).

The SAPK Hog1 interacts with elongating RNA Pol II (phosphorylated at serine 2 and 5 of the C-terminal domain), as well as with general components of the transcription elongation complex upon osmotic stress (Proft *et al*, 2006). In addition to its association with promoters, Hog1 is also present on coding regions of genes whose expression is induced upon osmotic shock and travels with elongating RNA Pol II (Pascual-Ahuir *et al*, 2006; Pokholok *et al*, 2006; Proft *et al*, 2006). It is worth noting that binding of Hog1 to coding regions is independent on the promoter bound-specific transcription factors but depends on the 3'UTR region of osmotic stress genes. The mechanism by which the SAPK is recruited to the 3' regions of osmotic stress gene remains unknown. By uncoupling Hog1-dependent transcription initiation from transcription elongation, it has been demonstrated that Hog1 at coding regions is essential for an increased association of RNA Pol II in ORFs, suggesting that it directly affects the process of elongation (Proft *et al*, 2006). Other yeast signalling kinases, such as Fus3 or PKA, have been reported to associate with coding regions of activated

Figure 3 The Hog1 stress-activated protein kinase (SAPK) regulates different phases of the transcription cycle. In addition to transcription initiation, Hog1 acts as a stress specific transcription elongation factor. Moreover, Hog1 targeting of the RSC complex displaces nucleosomes contributing to the efficient activation of transcription. The roles of Hog1 in other gene expression related processes such as mRNA processing, transport and translation are now starting to be elucidated.

genes (Pokholok *et al*, 2006), which indicates that signalling kinases have a role on transcription beyond initiation (Figure 3). Similarly, the *S. pombe* Sty1 SAPK also associates to the coding region of stress-responsive genes (Reiter *et al*, 2008).

Remodelling of chromatin in response to stress

The packaging of DNA into nucleosomes affects all phases of the transcription cycle from binding of activators and PIC formation to elongation. Thus, nucleosome positioning and dynamics is another layer of transcription regulation (Cairns, 2009; Jiang and Pugh, 2009). As in initiation, transcription elongation is affected by chromatin structure, which is regulated by several protein factors that covalently modify histones or temporarily move or disassemble and reassemble nucleosomes (Li *et al*, 2007a). Nucleosomes adopt canonical positions around promoter regions and more random positions in the interior of genes and are modulated to regulate DNA accessibility. Chromatin remodelling complexes utilize ATP hydrolysis to alter the histone–DNA contacts by unwrapping DNA transiently, forming DNA loops, sliding nucleosomes, displacing completely the histones from DNA or replacing histone subunits. The transcriptional stress responses and the chromatin structure are tightly linked (Uffenbeck and Krebs, 2006). Histone eviction may happen as a specific response to environmental stresses, leading to transcription reprogramming. For instance, it has been reported that nucleosomes are lost at several heat shock promoter sites within minutes of stress exposure (Shivaswamy *et al*, 2008).

Strikingly, in response to osmotic stress there is a dramatic change in the nucleosome organisation of stress responsive loci that depends on Hog1 and the RSC chromatin-remodelling complex. The RSC complex is a member of the SWI/SNF family and is characterized for modifying nucleosome structure through ATP hydrolysis. Upon stress, the SAPK Hog1 physically interacts with RSC to direct its association with the ORF of osmosensitive genes suggesting that this could be a major role for the SAPK during elongation. It is worth noting that in RSC mutants, RNA Pol II accumulates on stress-promoters but not in coding regions. RSC mutants also display reduced stress gene expression and enhanced sensitivity to osmotic stress. Cell adaptation under acute osmotic stress might, thus, depend on a burst of transcription that in turn could occur only with efficient nucleosome eviction. Two other chromatin remodellers have been associated with stress genes; the SWI–SNF complex, which is associated with stress-responsive promoters in response to stress (Proft and Struhl, 2002) and the INO80 complex (Klopf *et al*, 2009). Deficiencies in the INO80 chromatin remodelling complex results in extended expression of stress genes and a delay on nucleosome reassembly at stress-loci. Thus, a dynamic balance among different chromatin remodelling complexes seems to be required for proper expression of stress genes.

Control of mRNA processing, transport and translation by SAPKs

Eukaryotic mRNAs are synthesized by RNA Pol II as precursors that later on are strongly modified, spliced, cleaved at the 3' end and polyadenylated. In addition, export and translation of mRNAs is coordinated differently. At present,

it is still unclear whether stress activated genes are post transcriptionally regulated by specific mRNA binding proteins and the potential role of the SAPKs in regulation them.

The regulation of the stability of target mRNAs in response to different stimuli has been described to be one of the mechanisms used by SAPKs to control gene expression. p38 regulates the binding of the destabilizing factor tristetraprolin to AU-rich elements (ARE) in the 3'-untranslated regions of cytokine mRNAs, either directly or via the downstream kinase MK2 (Hitti *et al*, 2006; Ronkina *et al*, 2008; Sandler and Stoecklin, 2008). On the other hand, phosphorylation of KSRP, a KH domain RNA binding protein, by p38 regulates myogenic transcripts, linking SAPK activity and mRNA stabilization (Briata *et al*, 2005). Also, p38 stabilizes survival motor neuron (SMN) mRNA through the binding of RNA-binding protein HuR to the SMN ARE (Farooq *et al*, 2009). Similarly, p38 can also induce p21^{Cip1} mRNA stabilization, via phosphorylation of the RNA binding protein HuR, in response to ionizing radiation (Lafarga *et al*, 2009). In yeast, Sty1 controls mRNA stability of the transcription factor Atf1 through Csx1, a protein with three RNA recognition motifs (Rodriguez-Gabriel *et al*, 2003; Lawrence *et al*, 2007) and Hog1 regulates mRNA stability of the *TIF51A* and *MFA2* transcripts (Vasudevan and Peltz, 2001; Vasudevan *et al*, 2005). Recently, genomic wide scale analyses have shown that there are strong divergences between transcription rates and mRNA stabilities in response to osmotic shock in budding yeast (Molin *et al*, 2009; Romero-Santacreu *et al*, 2009). In a mild osmotic shock, stress-responsive mRNAs are specifically stabilized whereas most other mRNAs, like those for ribosomal proteins or cell wall components, are destabilized. It is clear from these reports that Hog1 has an effect on mRNA stability, especially in upregulated genes. However, the mechanism by which the SAPK Hog1 controls the stabilization of mRNAs is still unclear.

As other cellular stresses, osmotic shock too causes transient inhibition of translation initiation. Although the HOG pathway seems not to be involved in the initial inhibition of translation upon stress, this pathway is required for the recovery of translation initiation during adaptation (Uesono and Toh, 2002). Furthermore, activated Hog1 phosphorylates Rck2, a MAPKAP kinase family member that has been implicated in the regulation of translation (Bilsland-Marchesan *et al*, 2000; Teige *et al*, 2001). It has been also reported that Sty1 binds to translation factors and its mutation results in defects in the recovery of translation after stress (Asp *et al*, 2008). Therefore, SAPKs have an important role in protein production from mRNA biogenesis to translation.

Summary and perspectives

Stress-activated protein kinases have an essential role in transcription regulation by several unrelated mechanisms to assure the generation of a new transcriptional program upon osmotic stress. To start transcription initiation, Hog1 not only directly phosphorylates transcription factors, but also binds to chromatin having a more structural role. At the osmotic stress promoters, Hog1 functions as a platform to recruit the transcriptional machinery and histone-modifying complexes such as the Rpd3 histone deacetylase. Although it is clear that there are kinase-independent mechanisms in transcription

regulation, activity of Hog1 is needed to initiate transcription, at least for the recruitment of the SAPK onto the gene loci. However, it cannot be excluded that other targets exist that are phosphorylated by the SAPK during the initiation process. Of note, p38 and downstream kinases in the pathway can not only phosphorylate transcription activators but also the TATA-binding protein, chromatin-associated factors such as the nucleosomal proteins histone H3 and the non-histone chromosomal protein HMGN1. The identification of phosphorylation events mediated by Hog1 on the transcription machinery remains open. Once activated, Hog1 is also recruited to the coding regions of stress genes where it acts as a selective elongating factor that stimulates chromatin remodelling by RSC. How the SAPK is recruited to the coding regions is still unclear.

Eukaryotic cells have three main RNA polymerases that account for most of the nuclear transcription. RNA Pol I and Pol III are involved in the transcription of non-coding genes, such as those that encode tRNAs or rRNAs, whereas RNA Pol II directs the transcription of coding mRNA genes and many other ncRNA species. Recently, it has been reported that Sub1 (suppressor of TFIIB mutations 1) is involved in osmoregulation and in transcription modulation by both RNA Pol II and III (Rosonina *et al.*, 2009). Although both functions of this activator seem to be independent, it remains to be clarified whether SAPKs have a role in the regulation of RNA Pol I and III-dependent transcription.

The different mechanisms described for the yeast Hog1 SAPK in transcription modulation are exclusive neither for this kinase nor for unicellular organisms. It is worth noting that other MAPK family members as well as signalling kinases such as Snf1, Tor and PKA kinases also bind to chromatin. It should be noted that several reports support

an essential role of p38 SAPKs in the regulation of transcription upon inflammation and stress responses, as well as during skeletal myocyte, adipocyte, and cardiomyocyte growth and differentiation.

The generation of an adaptive program is essential to guarantee the maximal efficiency in cell survival in response to an immediate danger for the cell. The understanding on how Hog1 and related SAPKs in other organisms regulate transcription is illuminating how signalling kinases impinge on the control of gene expression. It also serves to define new mechanisms of regulation of such a complex multi-layered process. In addition, the knowledge of this regulation reveals which control steps are used by the cell to respond to extracellular stimuli once it requires a new and defined gene expression program within a very short period of time.

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Conflict of interest

The authors declare that they have no conflict of interest.

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