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STATE-OF-THE-ART REVIEW

Osmostress-induced gene expression – a model to understand how stress-activated protein kinases (SAPKs) regulate transcription

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Adaptation is essential for maximizing cell survival and for cell fitness in response to sudden changes in the environment. Several aspects of cell physiology change during adaptation. Major changes in gene expression are associated with cell exposure to environmental changes, and several aspects of mRNA biogenesis appear to be targeted by signaling pathways upon stress. Exhaustive reviews have been written regarding adaptation to stress and regulation of gene expression. In this review, using osmostress in yeast as a prototypical case study, we highlight those aspects of regulation of gene induction that are general to various environmental stresses as well as mechanistic aspects that are potentially conserved from yeast to mammals.

Stress responses

Environmental stresses include alterations in temperature, pH or oxygen concentration as well as nutrient deprivation, radiation or increases in the extracellular osmolarity. A critical property of living cells is their ability to sense and robustly respond to these fluctuations in their environment in order to maintain cellular functions. Thus, the immediate response to these stresses is crucial for cell survival, and is tightly controlled by multiple mechanisms. A common feature of many stress responses is the regulation of gene expression. Although the immediate role of gene expression in rapid adaptation is still being debated, it is generally held that gene expression is important for long-term adaptation to stress and for protection against future stress [1–5].

Changes in the environment trigger a large common transcriptional response, called the environmental stress response, in budding yeast (*Saccharomyces cerevisiae*), which is characterized by re-direction of resources from rapid proliferation to stress protection. In the environmental stress response, stress-induced genes include genes for defense against reactive oxygen species and DNA damage, carbohydrate metabolism and energy generation functions, whereas most stress-repressed genes have growth-related functions, such as translation and ribosome biogenesis [6–8]. Although environmental stress responses have common features, some aspects of the responses are unique to each individual stress. For instance, osmolyte-synthesizing

Abbreviations
lncRNA, long non-coding RNA; MAPK, mitogen-activated protein kinase; RSC, chromatin structure remodeling complex; SAPK, stress-activated protein kinase.

enzymes are specifically induced upon osmostress [9,10]. Transcriptional stress responses are sophisticated and fine-tuned; the magnitude and duration of the response is proportional to the severity of the perturbation, and different perturbations result in distinct expression signatures [7]. Stress responses are usually repressed or under tight control under non-induced conditions. Indeed, cells that display an increased basal transcription in response to environmental stress in the absence of stress show reduced fitness and slow growth phenotypes [11]. Correspondingly, sustained activation of stress responses leads to detrimental cellular growth or apoptosis [12]. This review highlights some of the emerging principles underlying the molecular events that trigger gene expression in response to osmostress, which is used as an example of a transcriptionally regulated response.

Signal transduction and the regulation of gene expression

Stressors activate a complex network of sensors and effectors from multiple signaling pathways that coordinate the required adaptive responses. An enormous effort has been made to understand the properties of these signaling pathways. One of the most intensely studied pathways is the high osmolarity glycerol (HOG) pathway, which was discovered 20 years ago, and is composed of membrane-associated osmosensors, an intracellular signaling pathway whose core is the Hog1 stress-activated protein kinase (SAPK), and cytoplasmic and nuclear effectors. Several studies have greatly advanced our understanding of the details of the architecture of the HOG pathway, and its specific regulatory properties have been reviewed elsewhere [10,13–16]. The emphasis here is on transcriptional mechanisms regulated by the Hog1 SAPK, for which emerging data are providing an understanding of how a signaling pathway rapidly, accurately and efficiently fine-tunes the full balance of a transcriptional program in response to extracellular stimuli [3,6,17,18]. As outlined below, this research in yeast is uncovering conserved principles in multicellular organisms that underlie regulatory strategies in response to changing environments.

The systems dynamics of the HOG signaling pathway have been recently studied by monitoring the behavior of single cells under controlled environmental conditions [19–21]. Signaling increases linearly, while transcription appears to be bimodal at low osmolarities. The transcriptional outcome measured by the expression of a fluorescent reporter under weak osmostress conditions is not continuously distributed, as seen by the presence of two distinct cell sub-populations: cells that are non-responsive and cells that fully express an osmoresponsive gene in response to identical Hog1 activation. In contrast, under stronger stress conditions, populations respond homogeneously (Fig. 1A) [22]. Chromatin structure appears to be one of the determinants of this stochastic expression, as a number of mutants in chromatin remodelers display altered bimodal responses (e.g. *gcn5* or *rsc9ts*) even at concentrations at which the transcriptional output of wild-type cells is uniform [22]. In addition, histone eviction (measured at the population level) at osmoresponsive genes is partial upon weak stress, suggesting that only a fraction of the population of cells remodels chromatin to allow efficient transcription. Interestingly, bimodal expression is not specific to osmostress, as oxidative and heat stress also result in bimodal expression, suggesting that bimodal behavior may be a general feature of stress-induced genes [22]. Based on these observations, several models have been developed to identify and predict the transcriptional output of osmostress stochastic gene regulation [23–25]. Thus, single-cell approaches may provide unbiased quantification of signaling/transcriptional outcome processes, and such approaches will be a powerful tool for defining new signaling features in response to stress in any type of cell and signaling pathway.

Global impact of stress on gene expression

Genome-wide studies have provided a global view of gene expression in response to osmostress. By following the kinetics of transcription over time in cells subjected to osmostress, a broader role of Hog1 as a master regulator of the massive transcriptional reprogramming that occurs was confirmed, and a relationship between the level of gene induction and dependence on the HOG pathway was suggested [7,26–28].

Recently, high resolution genome-wide approaches such ChIP-seq and tiling arrays have been instrumental in revealing the localization of the Hog1 SAPK, and of key components that drive osmoresponsive transcription, as well as in detection of new transcripts and complex transcriptional architectures [29,30]. In parallel with the induction of stress-responsive genes, a general phenomenon that occurs upon stress is a major down-regulation of general transcription that is possibly caused by a high rate of eviction of general transcription factors from chromatin caused by the osmotic imbalance [31]. In this scenario, Hog1 specifically targets RNA polymerase II to stress-responsive

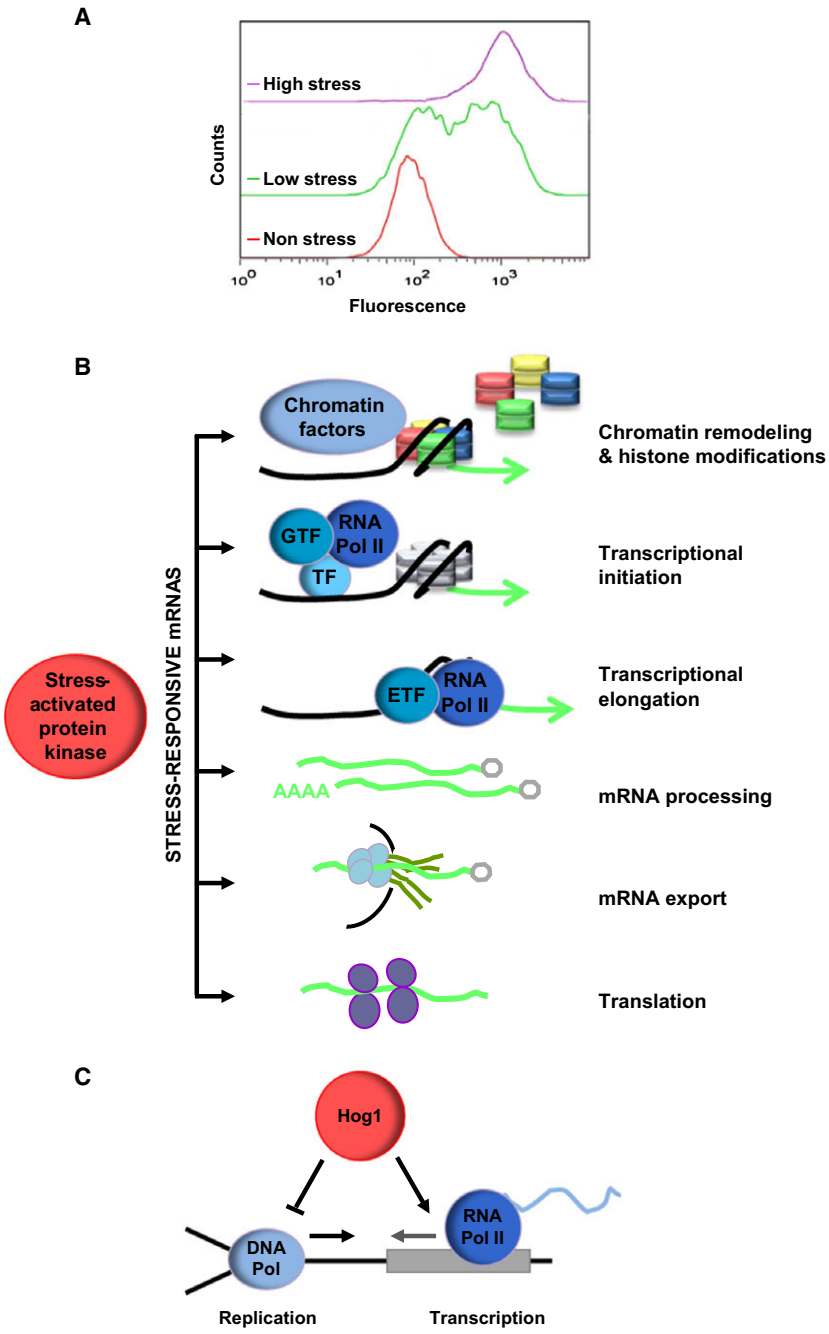

Fig. 1. Stress-induced gene expression by Hog1. (A) Schematic graph of bimodal expression of a fluorescent osmoresponsive reporter under low (0.1 M NaCl), high (0.4 M NaCl) and non-osmostress conditions, measured by single-cell flow cytometry. (B) Control of stress-responsive mRNA biogenesis by the Hog1 SAPK. Once activated, Hog1 associates with stress-responsive loci to modulate chromatin remodeling and histone modifications, transcriptional initiation and elongation. Hog1 also participates in mRNA processing, mRNA export and translation. (C) The Hog1 SAPK coordinates transcription with DNA replication. Upon osmostress, the activated Hog1 activates transcription and delays DNA replication by phosphorylating Mrc1. This coordinated regulation of the two processes prevents collisions between transcription and replication machineries and protects genomic integrity.

genes in order to bypass this general down-regulation of gene expression. This activity of Hog1 results in redistribution of RNA polymerase II away from other genes and towards those stress-responsive genes. Studies of Hog1 and RNA polymerase II co-localization showed that stronger binding of these proteins to chromatin positively correlated with maximal gene expression. Thus, it appears that there is a dedicated mechanism controlled by Hog1 that specifically targets gene induction under globally repressive conditions.

Association of signaling kinases with chromatin

Regulation of gene expression is a consequence of very fast signal propagation in response to extracellular stimuli. As mentioned above, a key observation that led to an understanding of how signaling translates into gene regulation was the observation that Hog1 associates with chromatin upon stress [32]. Additional mitogen-activated protein kinases in yeast such as

Fus3, Kss1 and Mpk1 have also been found to associate with chromatin [33,34]. Similarly, structurally and functionally unrelated signaling kinases, including Snf1 [35], Tor1 [36] and protein kinase A [33], have been reported to be recruited to chromatin. These findings suggest that kinases function as integral components of the transcriptional regulatory machinery.

In mammals, the Hog1 ortholog p38 also associates with chromatin [37,38], as do other mitogen-activated protein kinases (MAPKs) such as extracellular signal-regulated kinase and c-Jun N-terminal kinase [39–42]. Remarkably, the chromatin association of signaling components is not restricted to such kinases, but in some cases also includes their upstream activating kinases, such as mitogen-activated protein kinase 6 and meiotic kinase 1, or their downstream target kinases such as Msk1, or even their regulatory protein phosphatases (e.g. mitogen-activated protein (MAP) kinase phosphatase 1 phosphatase or calcineurin) [43,44]. These findings suggest a general model by which signaling complexes and their regulatory elements associate with chromatin as an additional layer of gene expression regulation.

Role of chromatin in gene regulation

Increased association of Hog1 with stress-responsive genes strongly correlated with chromatin remodeling and increased gene expression. Genome-wide nucleosome positioning by MNase-Seq analysis showed that, whereas genome-wide chromatin structure is not significantly altered upon stress, there is strong chromatin remodeling at stress-responsive genes that display Hog1 association [29]. The possibility that chromatin remodeling is an essential step for proper adaptation to extracellular stimuli is suggested by the fact that a number of mutants in the chromatin structure remodeling complex (RSC) are osmosensitive. Hog1 targets the RSC chromatin remodeling complex to stress-responsive genes, and RSC-deficient cells display reduced osmostress induction of gene expression [45]. External stimuli induce changes in genome-wide RSC occupancy that correlate with the induction or repression of specific families of genes that are regulated by stress [45–47]. In contrast, the INO80 chromatin remodeling complex appears to be important for reassembly of chromatin during adaptation [48]. The dynamic patterns of nucleosome positioning are central to repression of gene expression in the absence of stress and to establish a threshold for gene induction upon Hog1 activation [22].

In addition to chromatin remodeling, chromatin at stress-responsive genes is subjected to a number of modifications that establish a new landscape of histone marks [17]. A few studies have shown that, upon stress, several chromatin-modifying enzymes such as Spt-Ada-Gcn5-acetyl transferase complex (SAGA) histone deacetylases and methylases have an impact on stress-responsive gene expression [49–51]. For instance, the Rpd3 histone deacetylase is important for gene induction not only in response to osmostress but also other stresses [52,53]. Also, methylation of the histone H3K4 by the histone methyltransferase Set1 determines whether RSC or Swr1 complexes remodel nucleosomes at stress-responsive loci upon osmostress [54]. This observation suggests a new function for H3K4 monomethylation in governing the selectivity of chromatin remodelers for stress-responsive genes. Overall, based on examples of histone-modifying enzymes required for stress-responsive genes, it may be concluded that stress genes may be controlled in a manner that is distinct from the control of other housekeeping genes [3,41, 55,56]. Such control may be necessary due to the specific dynamics of induction and repression of these genes.

Multiple controls in mRNA biogenesis

Control of mRNA biogenesis may be exerted at various levels. Hog1 association with stress-responsive loci indicates that it has an intimate relationship with the transcriptional machinery. Indeed, Hog1 modulates several key steps during mRNA biogenesis (Fig. 1B and Table 1). Thus, initially Hog1 regulates initiation of transcription by (a) direct phosphorylation of specific osmostress transcription factors [57,58], (b) recruitment of RNA polymerase II and coactivators to osmoresponsive promoters [50,59], and (c) recruitment of chromatin modification and remodeling activities [29,45,51,60]. Hog1 also binds to the coding regions of osmoresponsive genes, where it acts as a transcription elongation factor specific for stress [61]. Remarkably, as described below, the role of Hog1 is not restricted to mRNA synthesis but is also important in modulation of additional aspects of mRNA regulation such as mRNA stability [62,63], mRNA export [64] and translation [65] in response to stress.

Changes in mRNA levels in response to environmental fluctuations are influenced not only by mRNA synthesis but also by mRNA degradation rates. The use of genomic run-on and dynamic transcription analysis assays has revealed that mRNA stability contributes specifically and dynamically to regulation of the gene expression program in response to osmostress [62,63,66]. Several recent reports have pointed out that the gene expression process may be 'circular', with the initial and final stages of the process being connected and mRNA levels being buffered by compensatory

Table 1. Factors involved in mRNA biogenesis and cell-cycle progression regulated by the Hog1 SAPK.

Factor	Reference
mRNA biogenesis factors	
RSC	[45]
Swr1 chromatin remodeling complex	[54]
SWI/SNF chromatin remodeling complex	[49]
SAGA	[49,50]
Rpd3 histone deacetylases	[51]
Set1 histone methylases	[54]
Smp1 transcription factor	[57]
Sko1 transcription factor	[58]
Hot1 transcription factor	[59]
Ubp3 ubiquitin protease	[60]
Nup1, Nup2 and Nup60 nuclear pore proteins	[64]
Rck2 kinase	[65,73]
Cell-cycle regulators	
Sic1 CDK inhibitor	[80]
Whi5 and Msa1 transcription factors	[82]
Hsl1 kinase	[85]
Mediator of replication checkpoint protein Mrc1	[88]

changes in mRNA synthesis and decay rates [67,68]. If this is the case in response to osmostress, there should be a transient link between transcription and mRNA degradation, and such a link may be controlled by the Hog1 SAPK.

Export of mRNAs from the nucleus is critical for efficient mRNA translation. In the initial minutes after stress application, general mRNA export appears to be slightly impaired, but stress-responsive mRNAs are still exported to the cytoplasm. Hog1 associates with components of the nuclear pore complex, and directly phosphorylates the Nup1, Nup2 and Nup60 components of the inner nuclear basket [64], suggesting that proper mRNA biogenesis of stress-responsive genes requires coordination of the action of synthesis and export machineries by the Hog1 SAPK.

Upon stress, there is a transient decrease in protein synthesis that is caused by a decrease in amino acid uptake, repression of ribosomal protein gene expression, and a decrease in translation efficiency [69–71]. The Hog1 SAPK does not appear to be involved in the initial inhibition of translation, but rather in reactivation of translation under stress, which functions as an adaptation mechanism [63,70,72]. The cytoplasmic Rck2 kinase, which is structurally homologous to mammalian calmodulin kinases, is directly phosphorylated and regulated by Hog1, and appears to be important for the regulation of translation [65,73].

Overall, a global picture is emerging in which, upon stress, RNA polymerase II accumulates at stress-responsive loci but its global accumulation at housekeeping gene loci is reduced. At the same time, Hog1 controls several aspects of mRNA biogenesis and protein translation of stress-responsive genes. This suggests the presence of a dedicated machinery for response to stress that is rapidly assembled to maximize the ability of cells to survive a sudden change in extracellular osmolarity. Similar dedicated processes may be assembled by other signaling kinases when cells are challenged with environmental changes that pose a risk to their survival.

Beyond mRNAs: lncRNAs

As described above, mRNA biogenesis is strongly regulated upon stress. However, the increased sensitivity and coverage of recent analyses have greatly expanded our understanding of the role of Hog1 in additional processes related to mRNA expression. For instance, new transcriptional roles have been identified for the Hog1 SAPK, such as modulation of RNA polymerase III-dependent genes [29], and the regulation of a novel class of functional long non-coding RNAs in response to osmostress [74].

Long non-coding RNAs (lncRNAs, > 200 nt long) have been identified in virtually all studied organisms regardless of genome size or complexity. However, our understanding of their properties is frequently only descriptive (i.e. size, stability and genomic location), and in most cases their function has not been assessed [75]. Expression of hundreds of lncRNAs is induced by the Hog1 SAPK upon osmostress [74]. The induction of a large number of lncRNAs upon stress is an additional layer of regulatory control that may affect gene expression and translation as well as enzyme function. For example, one gene that expresses a Hog1-dependent lncRNA in an antisense orientation is *CDC28*, which encodes the cyclin-dependent kinase 1 that controls the cell cycle in yeast. Cdc28 lncRNA mediates the induction of *CDC28* expression, and this increase in the level of Cdc28 results in more efficient re-entry of the cells into the cell cycle after stress. Thus, control of lncRNA expression is a mechanism for the regulation of cell-cycle progression in response to environmental stress [76]. Therefore, genome-wide expression and association analysis is expected to have a profound impact on the identification of new roles for SAPKs and signaling kinases in general in the regulation of gene expression.

Coordinated control of replication and transcription

The observed dramatic changes in gene expression that are mediated by Hog1 upon stress are coincident with

a delay in cell-cycle progression. Hog1 regulates multiple stages of the cell cycle by acting on core components of the cell-cycle machinery [77–79] (Table 1). For instance, Hog1 controls the G_1/S transition by down-regulating cyclin expression and stabilizing expression of the Sic1 cyclin-dependent kinase inhibitor [80–82]. Similarly in mammals, p38 down-regulates cyclin expression and phosphorylates the p57 cyclin-dependent kinase inhibitor during G_1 in response to osmostress [83,84]. Cells that are unable to delay cell-cycle progression upon osmostress display reduced viability under those conditions, both in yeast and mammals [80,83]. Thus, the regulation of cell-cycle progression is critical for maximization of cell survival upon stress. The Hog1 and p38 SAPKs are not only important for regulation of the G_1/S transition but also regulate other phases of the cell cycle such as the G_2/M transition in response to stress [77,85], suggesting that, in the presence of stress, cells need to delay the cell cycle to permit generation of adaptive responses before progressing into the next phase of the cell cycle.

Coordination between the cell cycle and transcription is even more necessary during S phase, where transcription needs to be spatially and temporally coordinated with DNA replication to prevent collisions between the transcription and replication machineries. Cells have evolved a number of mechanisms to ensure that both processes are compatible under normal growth conditions [86]. When yeast cells are stressed during S phase, Hog1 promotes gene induction, and, remarkably, also delays replication [87]. Hog1 affects early origin firing and fork progression by directly targeting Mrc1, a protein that links the Cdc45 helicase with DNA polymerase [88]. By delaying replication, Hog1 plays a key role in preventing conflicts between RNA and DNA polymerases (Fig. 1C). The phosphorylation of Mrc1 may be relevant not only for responding to osmostress, but also for coordination of DNA replication with any induced outburst of gene expression that occurs during S phase [77]. Thus, cells activate checkpoint surveillance mechanisms in response to extracellular stimuli to modulate cell-cycle progression and to permit adaptation to changing environmental conditions.

Conservation of the regulation of gene expression between yeast and mammals

The entire HOG SAPK pathway is conserved in higher eukaryotes, including humans, with the mammalian p38 SAPK being the structural and functional homolog of the yeast Hog1 SAPK [89,90]. p38 plays a key role not only in regulation of cellular responses to many types of stresses, but also in the regulation of proliferation, differentiation, survival and development of specific cell types [91]. A large body of evidence over recent years has highlighted that abnormalities in this pathway trigger pathological conditions, such as cancer, inflammation-related diseases and metabolic dysregulation [92,93]. Thus, we anticipate that the regulatory functions as well as the mechanisms of action of the HOG SAPK that have been identified in yeast may be relevant to understanding diseases related to SAPKs in humans.

It is clear that the p38 pathway has a pivotal role in stress-induced transcriptional responses. For instance, expression of between 60 and 88% of the genes induced in response to three distinct stress stimuli (tumor necrosis factor α, high osmolarity, and the protein synthesis inhibitor anisomycin) was dependent on the p38 SAPK [94]. Gene expression profiles changed, and the dependence on p38 decreased over time with stress [94–96], underlying the importance of p38 for the early transcriptional responses to stress. Indeed, the stress-induced genes were clearly enriched in transcription factors, suggesting that cells require an extensive program of gene expression for long-term adaptation to stress. Remarkably, only a small core set of key genes are commonly activated by all stresses. In addition, there are also differences in gene expression between cell types. Thus, it will be essential to elucidate gene expression patterns in specific cell types and in response to various stresses in order to fully understand the regulatory role of p38.

How does p38 regulate a gene expression program to allow cells to respond to specific stimuli? There has been intense research effort to answer this question [42,97,98]. Multiple mechanisms of transcriptional regulation have been described, focusing specifically on p38 and based on its homology with Hog1. As mentioned above, p38 associates with chromatin. p38 is recruited to chromatin via its interaction with specific transcription factors to ensure its specific localization in response to a stimulus [99,100]. Such binding of p38 allows recruitment of the RNA polymerase II machinery to trigger gene expression initiation [38]. Active p38 is also found within the coding region of the target genes and beyond, suggesting that p38 (similarly to Hog1 in yeast) travels with the RNA polymerase II machinery. How the p38 kinase is recruited to the stress-activated gene bodies, and what its mechanism of action is in transcriptional elongation, remain unclear in mammals. p38 also directly phosphorylates several transcription factors as well as chromatin modification and remodeling factors, resulting in alteration of their

protein stability or localization, or their affinity for DNA and protein partners. For example, p38 targets the myocyte enhancer factor 2D transcription factor by phosphorylation to allow recruitment of the myeloid/lymphoid or mixed-lineage leukemia-like protein methyltransferase complex, which trimethylates K4 of histone 3, to muscle-specific promoters during myoblast differentiation [101]. Another example is BAF60c, a subunit of the SWI/SNF complex, which is directly phosphorylated by p38 to allow chromatin remodeling and transcriptional activation of muscle-specific promoters [102]. Although our knowledge regarding the repertoire of p38 substrates related to transcription has increased over the years [98], it is clear that many unidentified p38 substrates remain.

Beyond mRNA biogenesis, p38 has also been found to regulate mRNA stability and translation [103,104]. p38 regulates the stability of various cytokine mRNAs, which involves mRNA elements such as AU-rich motifs, as well as mRNA-binding proteins such as tristetraprolin, HuR and hnRNPK homology-type splicing regulatory protein. For instance, kinases downstream of p38 regulate the stability of proinflammatory cytokine transcripts by phosphorylation of AU-rich element-binding proteins that interact with AU-rich elements in cytokine mRNA 3' UTRs [105]. Also, p38 acts on some mRNAs through regulation of the RNA binding protein HuR [106–108]. In addition to control of mRNA stability, p38 activation leads to rapid adjustment of protein synthesis. A known direct link of p38 to the translation machinery is the MAPK signal-integrating kinase Mnk. Upon activation by p38 MAPK, Mnk1 binds to eukaryotic initiation factor 4G and catalyzes the phosphorylation of eukaryotic initiation factor 4E [109,110]. All of these data clearly suggest that p38, similar to Hog1, is able to modulate several steps in mRNA biogenesis to modulate gene expression.

In summary, there is no doubt that SAPKs plays a central role in the regulation of chromatin and transcription in response to a stimulus. Using both genome-wide scale and context-specific approaches, together with mathematical modeling and single-cell analysis, it is expected that in the coming years we will learn more about how these SAPK pathways regulate gene expression, as well as the special characteristics of stress-responsive genes.

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Author contributions

EdN and FP wrote the manuscript.

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