MODES OF TRANSCRIPTIONAL REGULATION

Controlling gene expression in response to stress

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Abstract | Acute stress puts cells at risk, and rapid adaptation is crucial for maximizing cell survival. Cellular adaptation mechanisms include modification of certain aspects of cell physiology, such as the induction of efficient changes in the gene expression programmes by intracellular signalling networks. Recent studies using genome-wide approaches as well as single-cell transcription measurements, in combination with classical genetics, have shown that rapid and specific activation of gene expression can be accomplished by several different strategies. This article discusses how organisms can achieve generic and specific responses to different stresses by regulating gene expression at multiple stages of mRNA biogenesis from chromatin structure to transcription, mRNA stability and translation.

Exposure of cells to suboptimal growth conditions or to any environment that reduces cell viability or fitness can be considered stresses. Different types of stresses can be grouped into mild, chronic or acute stresses, which represent a dramatic shift in the environmental conditions. In this Review, we focus on these acute stresses, which require immediate and specific cellular responses for proper adaptation to maximize cell survival in response to extracellular changes.

Albeit to different extents, unicellular organisms and cells in multicellular organisms are exposed to constant changes in the environment that put them at risk. Thus, increases in oxidative stress, changes in external pH, nutrient supply, temperature changes or imbalances in osmolarity require adaptive responses for maximal cell survival. Adaptive responses depend on the organism, the natural environment in which it has been evolutionarily selected and its current physiological state. For example, unicellular organisms suffer from acute stress when they are exposed to sudden changes in temperature or in osmotic conditions, which will vary depending on the presence of water or highly concentrated solutes (such as sugars in fruits). By contrast, multicellular organisms have the capacity for internal homeostasis, thus they can more efficiently buffer extracellular changes to minimize intracellular alterations. However, even in multicellular organisms, particular cell types in specific tissues are exposed to sudden changes in the extracellular environment and thus they also have to be prepared to cope with those changes. Examples of such changes are the osmotic imbalances that are due to water availability in plant roots or the exposure to extremely high urea concentrations in mammalian renal cells.

Eukaryotic cells have evolved sophisticated sensing mechanisms and signal transduction systems that can produce accurate dynamic outcomes in response to stresses. Cellular stresses activate intracellular signalling pathways that control almost any aspect of cell physiology. Gene expression changes are a major component of stress responses, along with alterations in metabolism, cell cycle progression, protein homeostasis, cytoskeletal organization, vesicular trafficking and modification of enzymatic activities[1–9]. These responses are comprised of both generic responses that are shared by many stresses and specific adaptive responses that are dedicated to particular stresses[10–15]. Both general and stress-specific adaptive responses act over a series of timescales, from post-translational effects, which will provide immediate responses, to regulation of gene expression, which will be essential for the slower, long-term adaptation and recovery phases.

As observed in most adaptive responses, control of gene expression is tightly regulated and has fast response kinetics and controlled reversibility, which enables the cell to change its transcriptional capacity within minutes in the presence of stress and to return to its basal state after the stress is removed[16–19] (BOX 1). Recent genome-wide analyses of transcription, mRNA stability and protein association to chromatin, together with single-cell measurements, have provided a new picture of the molecular basis of gene expression regulation in response to stress. These new data sets are helping us to answer various questions, including the importance

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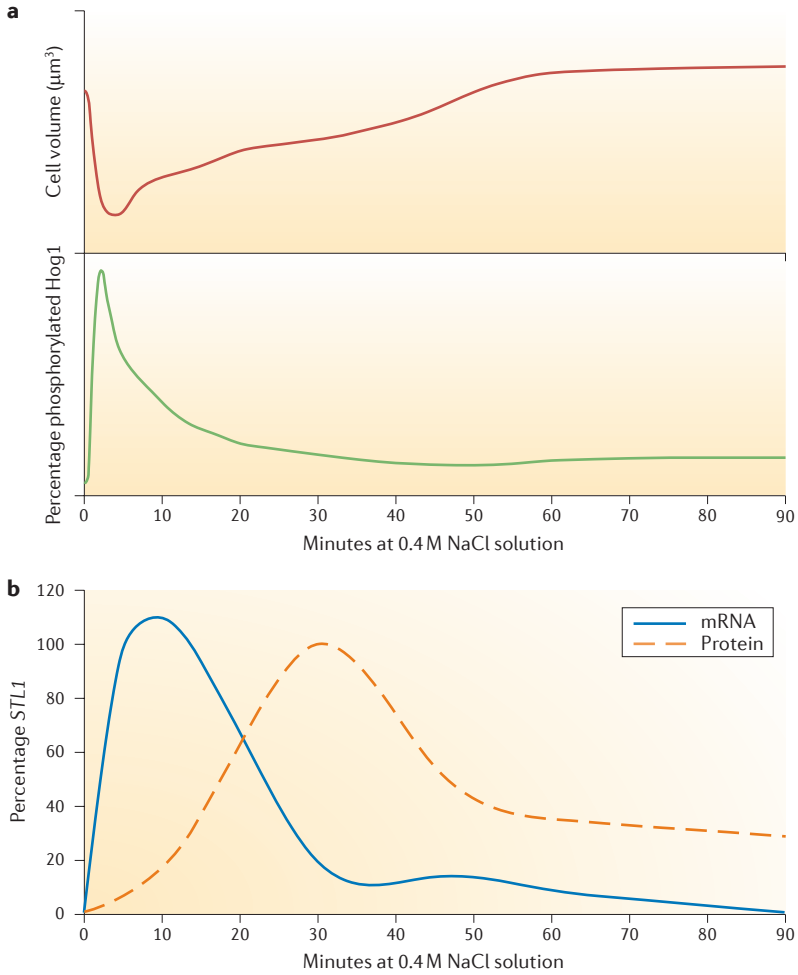
Box 1 | **Dynamic adaptive responses to osmostress**

Adaptation to environmental stress requires changes in many aspects of cellular behaviour. When subjected to increases in extracellular osmolarity, yeast cells rapidly lose water and shrink, and cell volume drastically diminishes within less than one minute (part **a** of the figure). Cells need to counteract these effects to maintain shape and turgor and to guarantee appropriate water and ion concentration inside the cell for optimal functioning of biochemical reactions. Thus, yeast cells accumulate small organic molecules, such as glycerol, which allow them to balance their osmotic pressure with that of the external environment. These osmolytes partly replace water, protect biomolecules and drive water back into the cell by osmosis[128]. Osmostress also has a great impact on cellular physiology, causing cytoskeletal reorganization, changes in cell wall dynamics, metabolic adjustments and cell cycle arrest, as well as causing modulation of transcription[6,13,14,129,130]. In *Saccharomyces cerevisiae*, the main molecule that is responsible for orchestrating all of this physiological change, as well as for controlling glycerol accumulation, is the p38-related stress-activated protein kinase (SAPK) Hog1 (REF. 131). Hog1 is rapidly but transiently activated by phosphorylation following exposure to stress (part **a** of the figure), and these activation kinetics correlate particularly well with cell volume changes. Of note, osmostress rapidly induces Hog1 nuclear accumulation[132,133]. Activation of Hog1 in response to osmostress is extremely transient because it is controlled by feedback mechanisms that make sure that the SAPK is inactivated during adaptation[50,134]. Modulation of gene expression in response to osmostress reflects the rapid and transient response of Hog1 activation and depends crucially on the severity of the osmotic stress. For instance, at 0.4 M sodium chloride (NaCl) solution, expression of stress-responsive genes such as *STL1*, which encodes a glycerol proton symporter, occurs within minutes of exposure to stress and protein production is achieved just 5–10 minutes later (part **b** of the figure). In addition to those general responses, a particular adaptive response to osmostress is the generation of intracellular osmolytes to balance internal with external osmolarity. This is important for mammalian epithelial or renal cells, as it is for yeast[135].



of gene expression changes relative to other cellular responses in stress adaptation, the mechanistic bases of the transcription processes regulated during stress, how stress signalling molecules influence chromatin structure and how multiple layers of control of different steps of mRNA biogenesis are coordinately regulated. Interestingly, addressing these questions might shed some light on the process of mRNA biogenesis in general, especially to understand transcription of genes that are subjected to sudden changes of activity.

In this Review, dynamic gene expression alterations that occur in response to stress are primarily illustrated using yeast and mammalian responses to acute osmotic shock (referred to here as osmostress) and the *Drosophila melanogaster* responses to acute heat shock (referred to here as heat stress). These systems underline some basic principles of stress responses that can help us broadly to understand many aspects of gene expression that occur in response to stress. We describe recent views of how, in response to stress, signal transduction pathways control gene expression by the coordinated regulation of several steps during mRNA biogenesis from chromatin dynamics, transcriptional initiation, elongation, mRNA modification, stability and export. Collectively, these processes permit rapid, coordinated and selective mRNA and protein production in response to stress.

Mechanisms of stress responses

Sensing. To handle the wide range of stresses that cells are exposed to, stress sensors are diverse and highly specialized. Furthermore, every organism has evolved a complete set of stress sensors that optimize cell survival in response to environmental changes. For instance, in yeast, osmostress is mainly sensed by two upstream mechanisms that converge on the high osmolarity glycerol (HOG) signal transduction pathway, which is the central pathway of the yeast osmostress response (FIG. 1). One sensing mechanism involves a 'two component' osmosensor that includes the osmosensing histidine kinase Sln1, which changes its activity depending on the membrane turgor (for example, REFS 20,21). These types of sensor are conserved in bacteria, *Dictyostelium discoideum*, fungi and plants, but they are not present in mammals[22]. In fact, except for ion channels, there is not a clearly defined mammalian osmosensor. A second mechanism involves highly glycosylated mucin-like transmembrane proteins, which are closely related to the mammalian mucins[23,24]. These sensors seem to be activated by conformational changes, and they have also been shown to be capable of activating signal transduction pathways in higher eukaryotic cells[25].

The underlying mechanisms for sensing heat stress are specific for this type of stress[2]. There are two main routes that are used to sense a sudden temperature change and to transmit the information. First is the evolutionarily conserved response to the heat-induced accumulation of denatured proteins[26], which results in the activation of the heat shock factor (HSF) transcription factor and the subsequent induction of heat shock protein (HSP) genes (see below). Second is the direct sensing of temperature changes through primary

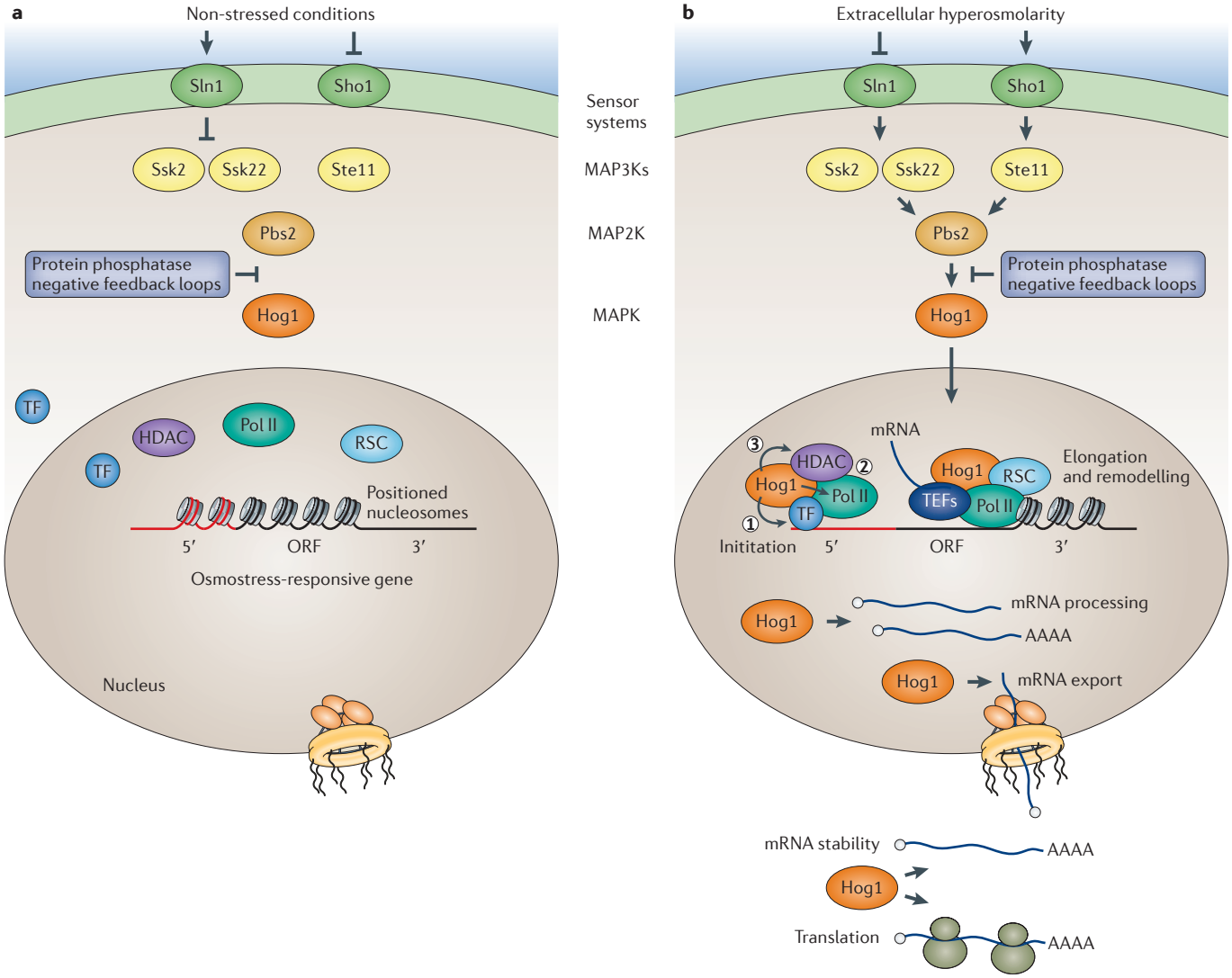


Figure 1 | **The HOG signalling pathway.** Stress-activated protein kinase (SAPK) pathways are signal transduction pathways that are required in response to stress. The HOG pathway is the yeast SAPK pathway. **a** | Under non-stressed conditions, the HOG pathway is not active and the Hog1 mitogen-activated protein kinase (MAPK) is mainly cytoplasmic. No expression of stress-responsive genes is observed. Hog1 remains dephosphorylated and inactive through the lack of relevant upstream signalling and the action of protein phosphatases and negative regulatory feedback loops. **b** | In response to osmostress, two independent upstream osmosensing mechanisms, the Sln1 and Sho1 branches, allow the activation of Ssk2, Ssk22 and Ste11 MAP3Ks, which, in turn, activate the Pbs2 MAP2K. Pbs2 combines both signals and activates Hog1, which accumulates in the nucleus and binds to the osmodependent promoters through specific transcription factors[41,82,87]. There, Hog1 may modulate initiation of transcription by direct phosphorylation of specific osmostress transcription factors (stage **1** in the figure)[80,81], recruiting RNA polymerase (Pol) II and co-activators to the osmoresponsive promoters (stage **2** in the figure)[61,79] and recruiting chromatin-modifying activities, such as the Rpd3 histone deacetylase (HDAC) (stage **3** in the figure)[74]. Hog1 also binds to the coding regions of osmoresponsive genes, acting as a transcription elongation factor (TEF) that is specific for stress[88]. Moreover, Hog1 displaces nucleosomes by targeting chromatin remodelling factors such as the RSC complex to these genes[64]. Hog1 is also involved in other gene-expression-related processes, such as mRNA processing[115,116] or translation[126], in response to stress.

Mucin
Mucins are a family of high-molecular-mass glycoproteins characterized by a high content of Ser and Thr residues that are organized as heavily glycosylated tandem repeats. Mucins are the main components of mucus, an adhesive and viscoelastic gel covering the surface of internal epithelia.

Thermosensory structures
Biomolecules that contain particular structures whose conformations are susceptible to temperature changes and behave as primary sensors of temperature. Examples of thermosensory structures include DNA, RNA, specific proteins or lipids from cellular membranes.

thermosensory structures, such as DNA, RNA, proteins and lipids, which either have a direct effect or lead to the activation of signal transduction pathways[1,27]. Examples of such thermosensory structures can be found from bacteria to mammals and include the alteration of DNA topology, the melting of RNA hairpins, the conformational change of certain proteins, such as Hsp26 (REF. 26), the modulation of histidine kinase activity in certain bacteria or the modification of ion-channel activation in the cell membranes of plants, *D. melanogaster* and mammals[28,29]. Overall, cells have evolved stress sensor systems — which are either membrane-bound or intracellular — that are specific to particular stresses and are responsible for the direct regulation of intracellular effectors or intracellular signalling pathways.

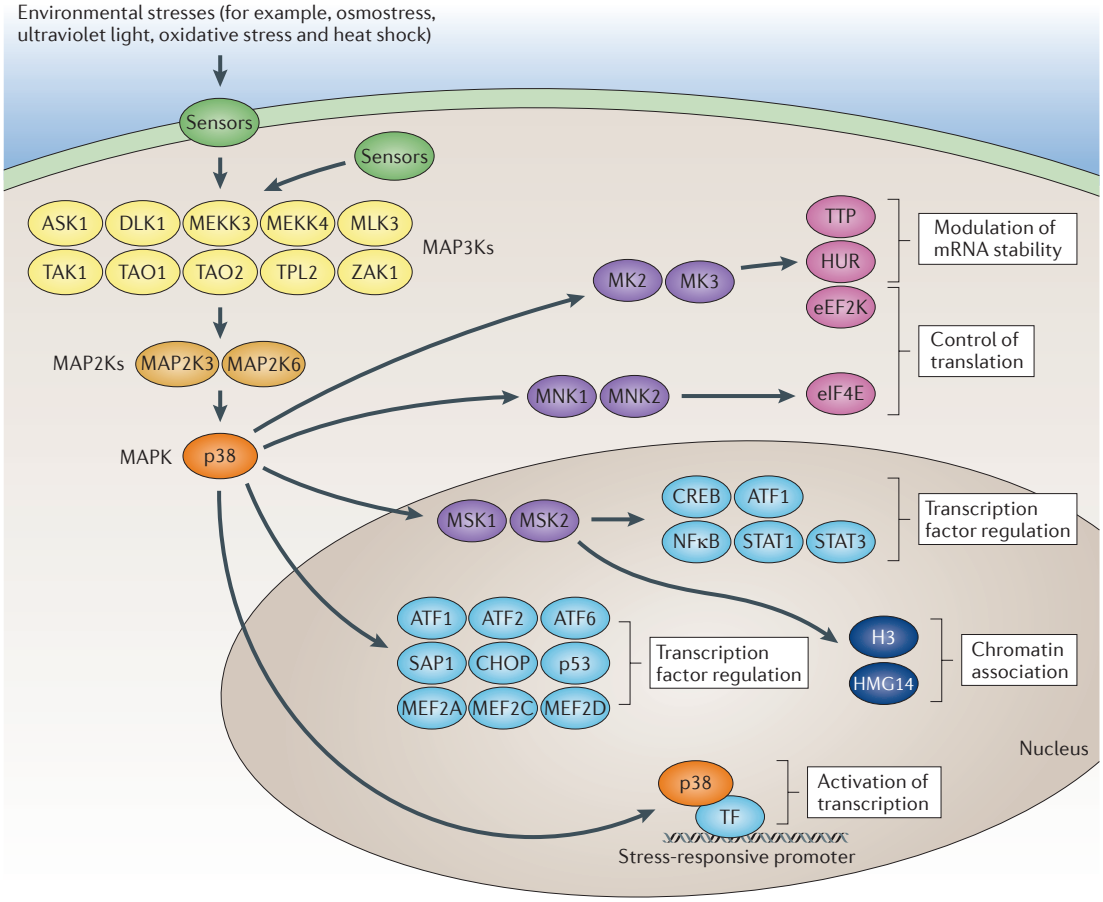


Figure 2 | **Mammalian stress signalling by p38 MAPKs.** Different environmental stresses, such as ultraviolet light, heat shock, osmostress or oxidative stress, as well as other stimuli, such as growth factors and inflammatory cytokines (not shown in the figure), can activate the mammalian stress-activated protein kinase (SAPK) pathway[32]. The p38 family of mitogen-activated protein kinases (MAPKs, shown in orange) are the mammalian orthologues of yeast Hog1. The family contains four isoforms (MAPK11–14) with many overlapping functions on the cell. In some cases, the stress sensors that regulate particular MAP kinase kinase kinases (MAP3Ks) have yet to be identified. Downstream targets of p38 MAPKs include several kinases that are involved in the control of gene expression and nuclear proteins, such as transcription factors (which are indicated by 'TF' in the figure) and regulators of chromatin remodelling. First, mitogen and stress-activated kinase 1 (MSK1) and MSK2 are activated by p38 and phosphorylate transcription factors (shown in light blue) such as CREB, ATF1, NFκB, STAT1 and STAT3, as well as the nucleosomal proteins histone H3 and the non-histone chromosomal protein HMG14 (REF. 137) (shown in dark blue). Second, MAPK-activated protein kinase 2 (MK2) and MK3 phosphorylate tristetraprolin (TTP), the RNA-binding protein HUR and eukaryotic elongation factor 2 kinase (eEF2K), which are involved in the control of gene expression at the post-transcriptional level[138,139]. Proteins with post-transcriptional effects on gene expression are shown in pink. Third, MAPK signal-integrating kinase 1 (MNK1) and MNK2 regulate protein synthesis by phosphorylating the initiation factor eIF4E[140]. Typical examples of phosphorylated transcription factors by p38 MAPKs in response to different stimuli are ATF1, ATF2, ATF6, SAP1, CHOP, p53, MEF2C and MEF2A[32]. As well as histone H3 phosphorylation by MSK1, other chromatin factors are directly modulated by p38 MAPKs. For instance, phosphorylation of the MEF2D transcription factor by p38 MAPKs is essential for histone H3 lysine modification[141]. Moreover, anchoring of active p38 MAPKs to target genes (for example, *FOS*, *PTGS2* and *IL8*) is mediated by the transcription factors ELK1, JUN and NFκB, respectively. Binding of p38 MAPKs to stress-dependent loci allows for recruitment of RNA polymerase (Pol) II and transcription[91].

Signal transduction. Cells need to integrate diverse inputs and initiate a rapid and appropriate response through signal transduction to the effectors. Signal transduction pathways serve to connect specific sensors that may be spatially restricted (for example, in the plasma membrane or in particular intracellular compartments) to target molecules that need to be activated for proper cellular adaptation. The signal transduction pathways in different organisms that are activated in response to different stresses are more conserved than sensing or effector mechanisms. Stress-activated protein kinase (SAPK) pathways are signal transduction pathways that are highly conserved in all eukaryotic cells from yeast to plants, insects and mammals and are responsible for responding to several stresses (FIGS 1,2). For example, the SAPKs in mammals are the

p38 mitogen-activated protein kinase (MAPK) family (which contains four isoforms, MAPK11–14); these signalling pathways are activated by a variety of environmental (physical and chemical) stresses, such as heat shock, ultraviolet light, gamma rays, anisomycin, osmostress and oxidative stress, as well as by physiological mediators, such as interleukins and tumour necrosis factor-α (TNFα)[30–32] (FIG. 2). Some, but not all, sensing mechanisms are known for these stresses. These stresses converge on the central core of p38 MAPKs but through different MAP kinase kinase kinases (MAP3Ks). Osmostress primarily induces activation of MEKK4 (also known as MAP3K4) and to a lesser extent other MAP3Ks, such as MEKK3 (also known as MAP3K3), ultraviolet light induces the activation of MEKK1 (also known as MAP3K1), and heat shock induces activation of ASK1 (also known as MAP3K5) and MEKK1. By contrast, TNFα induces activation of p38 MAPKs through the TNF-receptor-associated factor (TRAF) family of proteins and TAK1 (also known as NR2C2), which also mediates the activation of p38 MAPKs by cytokine receptors[32,33].

How specificity is obtained when a single MAPK pathway is activated by different stimuli is a challenging question. Cells have developed several mechanisms to achieve specificity and to increase the robustness of the pathway to alterations[34]: for instance, the use of scaffolds, the activation of accompanying specific signal transduction pathways (creating a network of responsive elements that are specific for each stimulus) together with a differential spatial and temporal activation of the pathway. (For example, particular MAP3Ks are activated by epidermal growth factor and osmostress on the plasma membrane, by anisomycin and ultraviolet light in the cytoplasm and by etoposide in the nucleus, as seen by MAP2K6 activation in single-cell analyses[35].)

Effector processes. Owing to the lag time of producing proteins *de novo* from stress-responsive genes, during the initial minutes of stress exposure, alternative, fast-acting responses are required. These fast responses include the downregulation of translation, the use of previously transcribed or translated proteins and the physical regulation of ion channels and transporters[7], and they usually depend on the type of stress. For instance, the Fps1 glycerol transporter is rapidly closed within seconds of exposure to osmostress in yeast[36], and Hog1 phosphorylates and activates the Na^+/H^+ antiporter (Nha1) and the Tok1 potassium ion channel[37]. Similarly, p38 MAPKs directly regulate Na^+/H^+ exchanger 1 (NHE1) in mammalian cells[32]. Therefore, this exemplifies the fact that there is a temporal program that occurs during stress, in which post-translational modifications will provide rapid defences. Then, post-transcriptional regulation (mainly regulation of translation and export of mRNA) will provide intermediate timescales, whereas regulation of gene expression will have a role after a few minutes of exposure to stress. These gene expression changes seem to be important for maximizing cell survival on exposure to subsequent stresses and for cross-protection against unrelated stresses[38].

Chaperones
Proteins that assist in the correct folding or assembly of other proteins.

Gene expression changes are an important component of the adaptive response to stress[39]. After the initial minutes of exposure to stress, there is a major change in the transcriptional pattern of the cell (see below). Heat stress leads to cell cycle arrest and, depending on the duration and severity of the heat stress, the accumulation of defects can lead to a reduction of cell viability[2]. The main defensive response to heat stress is characterized by a rapid increase of HSP chaperones, which maintain protein homeostasis, relieve folding defects and prevent protein aggregation and cellular damage[16,17]. In this response, induction of gene expression is absolutely required, as an increase in Hsp expression levels is essential to overcome protein and membrane alterations[2,3]. The physiological role of gene expression in cell survival during osmostress could have been overestimated or, at least, it is not completely understood. For instance, under mild stress conditions, a large segment of normal stress-induced gene expression seems not to be required, and the Hog1 kinase alone is sufficient to mediate cellular adaptation, perhaps by directly controlling the production of osmolytes[40]. Taken together, although the biological relevance of changes in gene expression depends on the organism and the strength and type of stress to which cells are subjected, regulation of gene expression is a major adaptive response to stress.

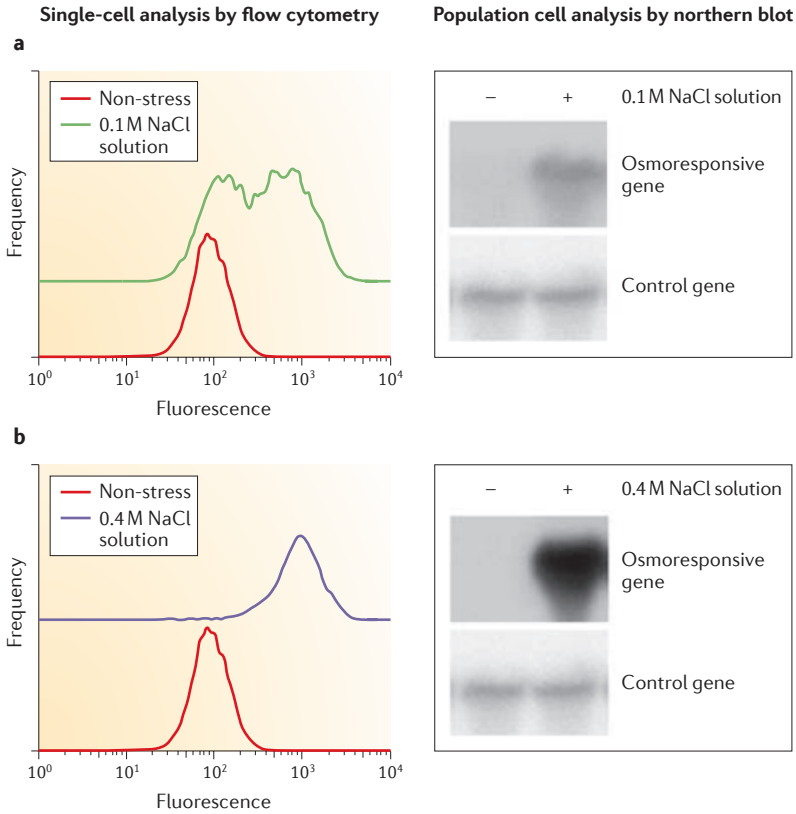
Stress-response kinetics

Ensuring a rapid response to stress. The speed of induction and the duration of the response are important parameters for optimal survival after stress. Usually, signal transduction mechanisms are activated within seconds of exposure to stress. How do signal transduction mechanisms enable these dynamics? The use of phosphorylation cascades is a conserved mechanism that has been harnessed to achieve this, as phosphorylation transduces signals much faster than systems that rely solely on gene expression. An example of a rapid phosphorylation event that occurs in response to stress is the activation of the yeast Hog1 kinase by phosphorylation in response to osmostress (BOX 1). Transcription of typical osmoresponsive genes, such as *STL1*, *GRE2* or *GPD1*, or heat-responsive genes, such as *HSP12*, *CTT1* or *ALD3*, is induced within 1 to 3 minutes in response to the respective stimuli of these gene inductions in yeast[17] (BOX 1). Indeed, more than 500 genes are transcribed in response to osmostress within the initial 10 minutes of stress[41–43]. In *D. melanogaster*, induction of heat-responsive genes is also observed within minutes[44]. Therefore, important changes in the transcriptional capacity of the cell, the extent of which depends on the organism, occurs within the initial minutes of exposure to stress and reflects the dynamics of the signal transduction process. Furthermore, the fact that multiple genes are synchronously activated is a clear indication that strong coordination is required to implement the proper gene expression regulation.

Temporal restriction of stress responses. Global adaptive responses need to be temporally restricted, as their constitutive induction is usually detrimental to cell growth.

Box 2 | **Single-cell studies**

Single-cell analyses have proved to be very useful for gaining insights into the variation of stress-responsive gene expression. These analyses — which use time-lapse microscopy together with fluorescently tagged molecules and transcription-specific reporters — have enabled gene expression studies to be taken beyond the reporting of the population average to the analysis of heterogeneity within a population of cells. Such studies have revealed that the gene expression output of the high-osmolarity glycerol (HOG) pathway has a 'bimodal' expression behaviour in mild stress conditions (0.1 M sodium chloride (NaCl) solution), as seen by the presence of two distinct cell subpopulations: non-responsive cells and cells that fully express an osmoresponsive gene (such as *STL1*) in response to identical Hog1 activation (part **a** of the figure)[73]. No induction of gene expression was detected in basal conditions, and all cells responded to 0.4 M NaCl solution (part **b** of the figure). In contrast to these single-cell studies, analyses by northern blot probing the same gene, which reports a cell population average, do not reveal the population distribution at 0.1 M NaCl solution but rather a slight induction of mRNA expression. Thus, single-cell studies can provide additional insights into our understanding of stress response within a population of cells. One of the determinants of this bimodal expression behaviour seems to be chromatin remodelling. Although measured at the population level (by chromatin immunoprecipitation (ChIP) analysis), histone eviction at osmoresponsive genes was partial at low stress levels, suggesting that only a fraction of the population could remodel chromatin to allow for efficient transcription. Thus, it seems that the partial histone eviction that is observed at the population level is responsible for the bimodal expression that is observed in single cells. Another determinant of the dynamic interplay between Hog1 activation and Hog1-driven gene expression is the retention time and concentration of Hog1 in the nucleus[73]. Remarkably, bimodal gene expression may be a general feature of yeast stress-induced genes, as it is observed in other stresses, such as oxidative or heat stresses[73]. Advances in live-cell imaging technology at the single-cell level in *Drosophila melanogaster* have also provided new insights into gene regulation. The recent emergence of this technology, together with fluorescence recovery after photobleaching (FRAP), allows the real-time imaging of transcription factors. Indeed, the chromatin structure and the dynamics of transcription factors at the inducible *Hsp70* loci in individual *D. melanogaster* salivary gland nuclei have been probed at high spatial and temporal resolution[71,103,136].



This cell growth defect is probably caused by the activation of cell cycle checkpoint systems and protective responses, as well as the energy diverted into the stress response from other cellular functions. For example, sustained activation of SAPKs, both in yeast and mammals, is detrimental to cell growth owing to induction of cell cycle delays and apoptosis-like responses (for example, REFS 45–47). Similarly, in *D. melanogaster*, induction of HSF activity is restricted within minutes of exposure to heat stress. When this strict temporal control is lost, such as during elevated HSF expression or by overexpression of transcriptional targets of HSF, cells suffer reduced fitness or even display compromised viability[48]. Therefore, temporal restriction of adaptive responses is important, and this is achieved by efficient downregulation of the inducers and signal transduction mechanisms that govern such responses. Although the timing is different for each cell type and stress, when cells start to adapt, there is a concomitant inactivation of the input signal to the signalling system and the downregulation of SAPK signalling pathways through dephosphorylation by phosphatases[49,50], as well as the presence of negative feedback loops controlling the signalling processes (for example, REFS 51–54). Mathematical modelling and single-cell studies of the HOG pathway in yeast have been very useful for illustrating some of the basic principles of SAPK signalling and the mechanisms of achieving dynamic and temporally restricted responses (BOXES 1,2).

Inactivation of the HOG pathway by transcription-independent mechanisms seems to be important for modulating acute responses, whereas transcription-dependent mechanisms might be important for proper adaptation to stronger stresses. HOG signalling inhibition may be constitutive in yeast cells. The HOG pathway is not just an 'on' and 'off' signalling system that is only activated in response to stress; rather, it is a system that is constantly 'on', but this basal level of HOG signalling is counteracted by an internal negative feedback that targets the Sln1 branch of Hog1 activation. This arrangement may allow higher efficiency in terms of faster response and fine-tuning of signalling thresholds on exposure to stress[55].

Thus, to maximize cell survival, signalling mechanisms and adaptive responses need to be highly reversible and fast-acting, a feature that is in need of a highly coordinated regulatory system.

The genomic landscape of stress-responsive genes

Although gene expression changes are only a part of the physiological responses to stresses, central questions are what the transcriptional responses of cells to stresses are and would this knowledge lead to the identification of the key survival factors? By addressing these questions, it might also be possible to understand how evolutionarily conserved the relevant strategies are and how important the transcriptional responses are for survival.

Global transcriptional responses to stress have been studied in detail using gene expression profiling in many organisms, including *Saccahromyces cerevisiae*, *D. melanogaster* and mammals. In yeast, approximately

600 genes are downregulated in response to several stresses (for example, osmostress, heat shock, oxidative stress or nutrient deprivation) that are involved in growth-related processes, RNA metabolism, protein syntheses and genes encoding ribosomal proteins[56,57]. Conversely, there are a large number of genes, up to 300–400 genes, that are strongly induced on exposure to those stresses[16,41,56]. This number increases up to more than 1,000 genes when, for instance, mildly induced genes are considered[43]. Exposure of mammalian cells to several stresses, such as heat shock and oxidative stress, for 30 minutes induced around 100 genes[58], and exposure to osmostress, TNFα and anisomycin for only 45 minutes also induced the expression of more than 120 genes that were mostly dependent on the p38 MAPKs[59].

Those genes that are similarly regulated in response to several stresses are part of the environmental stress response (ESR). ESR includes genes that are involved in carbohydrate metabolism, transport and detoxification, molecular chaperones, protein metabolism, intracellular signalling and DNA repair, and this response has been related to cross-protection[38]. The extent of the ESR might represent up to 80% of all genes that are regulated by a specific stress and its severity, as increasing extremes of stress usually lead to greater changes in gene expression[17,41]. In addition to the ESR, there are a number of genes that are specifically expressed for each type of stress. In contrast to the situation in yeast, the common stress response in *D. melanogaster* and mammals is much more restricted. For instance, in *D. melanogaster*, approximately 200 genes are rapidly upregulated in response to heat stress, but only around 70 of those are also responsive to other stresses[18]. Similarly, in mammals, ~100–150 genes are rapidly upregulated on exposure to heat stress, osmostress or oxidative stress, but the common response is restricted to approximately 30% of them. Interestingly, a large number of those common genes encode transcription factors. Moreover, here, different cell types display different transcription patterns to stress[58,59].

Stress-essential genes are those that are required for adaption to a particular stress. As for stress-induced genes, they encompass almost all general cell features, from metabolic adjustment (carbon and energy metabolism) to mRNA synthesis, cell-type differentiation, cellular transport and cytoskeleton organization[60–63]. In certain cases, induction of a particular gene is required for adaptation to stress (such as the induction of glycerol-3-phosphate dehydrogenase (*GPD1*) for adaptation to osmostress in yeast). However, there is generally a low overlap between those genes that are transcriptionally induced in response to stress and those genes that seem to be essential for adaptation. Possible explanations for this discrepancy are that stress-essential genes may be more relevant for long-term adaptation or for subsequent stresses (rather than being crucial for the immediate response to stress), or that it is the network of induced genes (rather than the effect of a particular single gene) that is relevant for cell survival and adaptation to stress.

Fluorescence recovery after photobleaching
(FRAP). An optical technique for quantifying the kinetics of diffusion or active movement of biological molecules. This method involves labelling a specific cell component with a fluorescent molecule, followed by photobleaching a sharply defined region of the cell. Imaging is used to observe the subsequent rates and patterns of fluorescence recovery.

SWI/SNF
A chromatin-remodelling complex that uses DNA-dependent ATP hydrolysis to mobilize nucleosomes and render the DNA accessible for various nuclear processes. The SWI/SNF complex is required for expression of many inducible genes.

Control of mRNA biogenesis under stress

The inducible gene expression kinetics observed in response to stress is achieved by fine regulation of multiple steps of the mRNA biogenesis process. Although this is common to many stresses, the underlying mechanistic details of how such regulation is achieved are highly dependent on the particular stress and organism. This complexity poses the questions of what the advantages of such a complex regulatory network are and how this coordinated control is achieved.

Chromatin remodelling and modification. The packaging of DNA into nucleosomes affects all phases of the transcription cycle, and thus nucleosome positioning and dynamics are key layers of transcriptional regulation. Chromatin structure is regulated both by chromatin remodellers that move, disassemble or reassemble nucleosomes and by factors that covalently modify histones; recruitment of chromatin remodellers and modifiers to stress-responsive genes can allow transcription factor accessibility at RNA polymerase (Pol) II promoters.

There is evidence that several chromatin remodellers can be recruited to promoters during stress responses. For example, during osmostress in yeast, there is a dramatic change in the nucleosome organization of stress-responsive promoters that depends on Hog1 and the RSC chromatin-remodelling complex[64]. Another chromatin-remodelling complex, SWI/SNF, is recruited to stress-dependent target promoters in a manner that correlates well with transcriptional induction of target genes. Increased SWI/SNF association at promoters under osmostress is abolished in a *hog1*-null strain[65]. Also, during heat stress in yeast, nucleosomes are evicted or displaced from heat-stress-dependent promoters during activation of transcription and the chromatin-remodelling complexes SWI/SNF, ISW1 and RSC seem to have partially overlapping functions in this process (for example, REFS 66–68).

In addition to altering promoter accessibility, chromatin dynamics occurring in coding regions in response to stress has been studied. During the elongation phase of transcription, RNA Pol II must contend with nucleosomes that act as barriers. For example, in response to osmostress in yeast, nucleosome organization within stress-responsive genes is altered in a Hog1-dependent manner[64]. A dynamic balance among different chromatin-remodelling complexes is required for proper expression of stress genes, as chromatin remodelling can be involved in switching genes 'off' as well as 'on'. For example, deficiencies in the INO80 chromatin-remodelling complex result in prolonged expression of stress genes and a delay of nucleosome reassembly at stress loci[69], thus, this chromatin-remodelling complex is responsible for nucleosome repositioning, which limits the extent of transcription induction.

Unlike yeast, in which promoters contain a positioned nucleosome within the first 100 bp of the transcription unit, some *D. melanogaster* genes, such as *Hsp70*, that are associated with a paused polymerase (see below) contain a nucleosome-free region that extends further downstream; the first nucleosome is centred 330 bp

after the transcription start site[70]. Chromatin architecture throughout the *D. melanogaster Hsp70* genes has an initial dramatic change in response to heat stress, a change that it is independent of transcription, followed by a second disruption of nucleosome structure that is transcription-dependent[71]. The initial nucleosome loss is necessary but not sufficient for optimal transcription of heat-shock genes, suggesting that this might prepare genes for optimal gene expression[72]. Similarly, recent single-cell studies of gene expression in yeast on exposure to osmostress have shown that full induction of expression depends not only on signalling but also strongly depends on the presence of nucleosomes in those genes[73]. Therefore, nucleosome remodelling can be important in providing transcriptional activators and general transcription machinery with full access to stress-responsive genes.

Complementing the role of nucleosome remodellers, recruitment of histone-modifying enzymes provides another means of modulating transcription. Some covalent histone modifications have been coupled to stress-dependent changes in gene expression in yeast. The Rpd3 histone deacetylase complex is recruited by Hog1 to specific stress-activated promoters following stress[74]. Other stresses, such as heat shock and oxidative stress, also require Rpd3 for full activation of gene expression[57]. Although histone deacetylation is usually associated with transcriptional repression, in stress responses it is required for transcriptional activation and might serve to recruit additional factors that are required for full gene expression. In addition, other post-translational chromatin modifications are associated with the heat stress response. For example, in *D. melanogaster*, DNA sequences that are destined to be strongly bound by *D. melanogaster* HSF after heat stress are associated with distinct chromatin marks, such as histone mono-ubiquitylation of H2B and H3K4 trimethylation, compared to sites that are unoccupied by HSF[75]. Genetic analyses have shown that multiple histone residues, when mutated, prevent modifications and render cells sensitive to different cellular stresses[76], indicating that several chromatin-modifying complexes and histone modifications might be important for transcription initiation on exposure to stress.

Control of mRNA synthesis. At least two different mechanistic approaches have been identified by which similar dynamic transcriptional responses to stresses are achieved. The first, which is exemplified by several stress responses in yeast, involves appropriate recruitment of transcription factors, including RNA Pol II, to affect initiation, elongation and mRNA stability[13,14]; in the case of osmostress, the recruitment of these factors is orchestrated by Hog1. In the second, which is exemplified by *D. melanogaster* heat stress response, the basic transcriptional machinery is pre-assembled at the stress-responsive genes in non-stress conditions, and it is the binding of HSF and its phosphorylation at these promoters that leads to rapid induction of gene expression[11,15]. Both mechanisms involve an initial regulation of specific transcription factors.

To study the first mechanism, the genome-wide occupancies of various transcription-related proteins and how these patterns are altered in response to stresses have been dissected by chromatin immunoprecipitation (ChIP)-binding analyses. Combined with global gene expression profiles from mutant yeast strains, these approaches have revealed that a complex transcriptional network that has different contributions among transcription factors and specific binding dynamics operates following the exposure of yeast to osmotic stress[41,42] and other stresses[68,77,78]. The transcription factors are regulated in several ways, including by modulation of their nuclear localization, their recruitment to specific stress-responsive genes and/or their activity[43,79–81].

Interestingly, in addition to regulating transcription factors, signalling kinases have now been shown to interact with chromatin. In yeast, kinases such as Hog1 and the MAPKs Fus3, Kss1 and Mpk1 or unrelated signalling kinases, including Snf1, are recruited to chromatin[82–84]. Furthermore, binding of signalling kinases to chromatin has been shown to occur in higher eukaryotes[14]. These data suggest a novel and widespread role for signalling kinases in chromatin regulation[85,86]. For example, in yeast, Hog1 can act directly at chromatin by interacting with transcription factors[82,87–89] at promoters, where it mediates the recruitment and activation of the basic components of the transcriptional machinery and chromatin modifiers such as RNA Pol II, Mediator, SAGA, SWI/SNF, Rpd3 and the Ubp3 ubiquitin protease[61,65,74,79,90]. Indeed, even when it is artificially tethered to promoter DNA, Hog1 can activate transcription[79], emphasizing that the association of Hog1 with target promoters has a crucial role in the stimulation of gene expression. Of note, binding of Hog1 is not restricted to promoters but also occurs at coding regions, where it is essential for an increased density of active RNA Pol II in the coding regions[88], thus suggesting a role for Hog1 during transcriptional elongation. An example in mammals is the binding of the p38 MAPKs to stress-responsive loci (promoters and coding regions) on exposure to several types of stresses[91]. Similar processes may occur in non-stress situations; during skeletal myogenesis, MAPK14 (also known as p38α MAPK) is recruited to chromatin and targets the SWI/SNF chromatin-remodelling complex to muscle-regulatory elements, possibly by MYOD and/or its partner E47[92,93]. Similarly, ERK1 (also known as MAPK1) and its target mitogen and stress-activated kinase 1 (MSK1; also known as S6Kα5) are also recruited to genes in response to progesterone[94]. Interestingly, binding of ERK1 or MAPK14 to the *FOS* promoter is mediated by the ELK1 transcription factor[91,95].

RNA Pol II pausing is a mechanism that enables rapid gene induction and is widespread in developmental control and environmental response genes; it has been studied extensively in flies but is also reported in human cells[96–101]. In *D. melanogaster*, heat-shock-regulated genes in non-stressed cells are already bound by RNA Pol II, but the polymerase is paused after transcribing 20–40 nucleotides[101–103] (FIG. 3). Following exposure to stress, escape of the paused polymerase requires recruitment and activation of HSF. The monomeric form of HSF is

Chromatin immunoprecipitation
(ChIP). A method used to determine whether and where a given protein associates to DNA. This technique is also used to characterize the distribution of specific chromatin marks on the genome.

Mediator
A ~30-subunit co-activator complex that is necessary for successful transcription of class II promoters of metazoan genes. Mediator coordinates the signals between enhancers and the general transcription machinery through its interaction with RNA polymerase II and site-specific factors.

SAGA
The yeast SAGA complex (Spt–Ada–Gcn5–acetyltransferase) is a large, multi-subunit complex containing several enzymatic activities that are linked to activators and histones and involved in core promoter selectivity. SAGA is necessary for turning on genes that respond to stress. It shows a high degree of structural conservation with a human complex: the TATA box binding protein (TBP)-free TAFII-containing complex.

FOS
An oncogene that is activated by diverse stimuli and stresses, including serum growth factors and MAPK cascades. Members of the FOS family can dimerize with JUN proteins to form the activator protein 1 (AP1) transcription factor, which has been involved as a regulator of cell proliferation, differentiation and transformation.

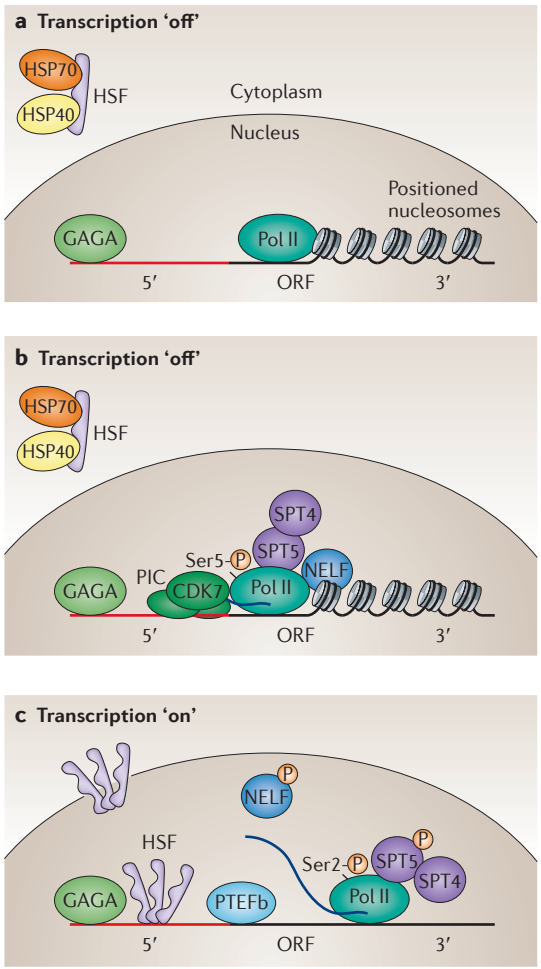


Figure 3 | **Control of gene expression by the HSF transcription factor in *Drosophila melanogaster*.** **a** | In non-stress conditions, heat shock factor (HSF) is maintained in an inactive monomeric form by association with the heat shock protein 70 (HSP70) and HSP40 chaperones in the cytoplasm. Moreover, RNA polymerase (Pol) II and the GAGA factor are already associated with heat-dependent promoters, such as at the *Hsp70* loci[102]. **b** | Then, also before heat-shock activation, the GAGA factor recruits co-activators, the general transcription factors and nucleosome remodelling factors, triggering pre-initiation complex (PIC) formation at the promoter. Then, the cyclin-dependent kinase 7 (CDK7) subunit of TFIIH phosphorylates Ser5 of RNA Pol II carboxyl-terminal domain (CTD), and RNA Pol II initiates transcription into the first 20–40 bases of the gene. RNA Pol II remains paused by the negative elongation factor (NELF) and DRB-sensitivity-inducing factor (DSIF), which is composed of SPT4 and SPT5[142–145]. **c** | In response to heat stress, HSF trimerizes and is transported into the nucleus, where it is subjected to various post-translational modifications and binds to heat-dependent promoters[2,10]. HSF recruits additional co-activators, such as PTEFb (which consists of CDK9 and cyclin T), and factors with nucleosome remodelling activities. PTEFb then phosphorylates Ser2 of RNA Pol II CTD, SPT5 and NELF, triggering NELF dissociation from Pol II and releasing RNA Pol II from its paused state into productive transcription elongation[106]. Note that heat shock induces a rapid loss of nucleosomes across the heat-dependent gene that precedes transcription and is independent of it, but is dependent on HSF, GAGA and PARP[71] (not shown in the figure).

inactive, and its conversion to an active, high-affinity DNA-binding form involves HSF trimerization, a common feature in all eukaryotic HSFs[10]. Under non-stress conditions, HSF monomers associate with multiple HSPs; on stress exposure, HSF dissociates from the complex, homotrimerizes and binds to DNA[104]. The control of HSF by HSPs provides an important control step that monitors the levels of free chaperones in the cell. Although HSF1 is conserved among eukaryotic cells, the mammalian HSF family consists of four members with unique and overlapping functions that have selective tissue-specific characteristics[105]. HSF is regulated not only by its binding to DNA but also by post-translational modifications such as phosphorylation (for example, by p38 MAPKs), sumoylation or acetylation and by interacting protein partners. This is a clear indication of the existence of direct input signals that modulate HSF activity in addition to its regulation by HSPs[10,12,15].

Binding of HSF to DNA is required but is not sufficient to activate transcription of heat-responsive genes. HSF mediates the recruitment of a second factor, positive transcription elongation factor b (PTEFb; which consists of cyclin-dependent kinase 9 (CDK9) and cyclin T (CYCT)) that phosphorylates the carboxyl-terminal domain (CTD) of RNA Pol II to activate transcription[106]. PTEFb also phosphorylates negative elongation factor (NELF) and transcription elongation factor SPT5, thus alleviating the inhibitory effects of these factors on the polymerase[100,107], releasing it into productive transcription elongation[101,103,108]. Recent technological advances in microscopy now allow for real-time kinetic analyses of transcription factor recruitment to an actively transcribing locus. In *D. melanogaster*, the transcription factors follow a sequential order of recruitment to the heat-activated *Hsp70* loci, and this process occurs synchronously within a population of cells[103].

The fact that heat-responsive genes in yeast do not have paused polymerase and yet are still rapidly expressed opens the possibility that yeasts have alternative mechanisms to achieve a similar response. The possible need for a different mechanism could reflect the simpler genomic structure and more compact gene organization of yeast or that the need for coordination is not as important as it is in complex organisms. Actually, several lines of evidence indicate that the role of this paused polymerase in multicellular organisms might be to facilitate a faster initial response to stress and also to permit synchronous gene activation[95]. Of note, and consistently with heat-responsive genes in *D. melanogaster*, stress-responsive genes in mammals,

Sumoylation
The post-translational modification of proteins that involves the covalent attachment of a small ubiquitin-like modifier (SUMO) and regulates the interactions of those proteins with other macromolecules.

such as *FOS* or *MYC*, contain paused RNA Pol II[109]. However, in response to stress, those loci recruit signalling kinases and there is a substantial increase in RNA Pol II occupancy[91]. Therefore, in mammals, restarting paused RNA Pol II, as well as recruiting signalling kinases and additional RNA Pol II, could enable a fast and coordinated response system.

mRNA stability. Nascent RNA transcripts undergo splicing, nuclear export, stabilization and translation; control of such RNA processing is another layer of regulation of gene expression in eukaryotic cells that is used in stress responses. The regulation of the stability of target mRNAs in response to different stimuli is another mechanism by which SAPKs control gene expression. For instance, p38 SAPKs regulate the binding of the destabilizing factor tristetraprolin (TTP) to AU-rich elements (ARE) in the 3′UTRs of mammalian cytokine mRNAs, either directly or by the downstream kinase MAPK-activated protein kinase 2 (MK2; also known as MAPKAPK2)[110,111]. Also, several p38 MAPK family members act on the mRNAs encoding survival motor neuron (SMN) and $p21^{CIP1}$ through regulation of the RNA-binding protein HUR[112,113]. Changes in mRNA stability also occur under heat shock[114]. In budding yeast, there are global changes in transcript stability occurring in response to osmostress that make substantial contributions to the changes in the steady-state mRNA levels during stress. Although changes in mRNA stability, especially in up-regulated genes, are dependent on Hog1, the specific mechanisms are currently unknown[43,115,116].

It is thought that sophisticated regulatory mechanisms are involved in modulation of mRNA stability, as stress-responsive mRNAs are independently either selectively stabilized or selectively degraded from global mRNAs; both the induction and decay rates are regulated separately and the regulation alters depending on the phase of response to stress[43,116,117].

Control of translation. In response to stress, there is a transient decrease of the production of growth-related proteins, whereas the production of stress-related proteins increases. Control of gene expression at the level of translation is particularly valuable for an organism because the translation of existing transcripts generates proteins more rapidly than with *de novo* transcriptional induction, and thus this might be a relevant initial adaptive response to stress. This typical stress response is observed from yeast to mammals, and it is clearly illustrated by the heat shock response in mammalian cells[118]. Concomitantly with the induction of HSP gene transcription, there is an increase in the efficiency of HSP mRNA translation, as well as a general decrease in the global rates of protein synthesis. This is achieved, at least in part, by the coordinated effect of several aspects; for example, through the regulation of several translation factors, such as eukaryotic translation initiation factor 4E (eIF4E), eIF2a and eIF4G family members. Some of these factors are directly controlled by HSPs. Additional translational regulation occurs by the spatial control of translation in stress granules (for example, REFS 119–123).

Similarly, in yeast, despite a global reduction of translation efficiency following exposure to stress, some stress genes are translated more efficiently[124]. Hog1 is required for the fast recovery of translation initiation on exposure to osmostress[125] through the regulation of the SAPK Rck2, which phosphorylates the translation elongation factor EF2, and this is required for translational efficiency in response to osmotic stress[126,127]. Interestingly, Hog1 has a key role in the translational response to osmostress, thus highlighting the importance of translational control for fine tuning of the adaptive responses[124].

Conclusions and future directions

Proper adaptation to stress is crucial for cell survival in harsh environments, and cells have developed sensing and signal transduction mechanisms that permit the appropriate adaptive responses[25]. Usually, one important part of the different adaptive strategies consists of a massive reorganization of the gene expression programme[13–15].

The studies in yeast and *D. melanogaster* have been instrumental in understanding basic molecular mechanisms that mediate stress-regulated gene expression. However, all organisms respond to stress and require adaptive responses to survive. Although some aspects of the stress responses may be species-specific, the conservation of some of the regulatory mechanisms — such as the binding of signalling kinases to chromatin and the presence of paused RNA Pol II at the promoters of certain genes — are key indications that data from model organisms will continue to enhance our understanding of gene expression in higher organisms.

Control of gene expression on exposure to stress mirrors some of the basic properties of the signal transduction pathways that respond to environmental changes. Therefore, despite the focus of many studies on individual transduction pathways, it has to be expected that an integrated signalling network ultimately determines gene expression. Genome-wide studies have shown that signal transduction pathways control a variety of downstream elements that permit a rapid change in the transcriptional landscape of a cell within minutes of exposure to stress[41,42]. However, many things still need to be uncovered to understand the molecular bases that determine the main characteristics of the dynamic response of stress-responsive genes. For example, we currently lack a detailed, functional understanding of chromatin modifications and their relationship to the plethora of factors that associate with stress-responsive genes. Moreover, our knowledge of the identity and functions of the transcription factors that are relevant for stress-induced gene expression is incomplete. Some of this information should come from systematic integrated data sets on transcription, protein binding, protein modifications and protein interactions. Such comprehensive data may help us to understand the *cis*-regulatory characteristics of the genes that are induced versus those that are repressed in response to stress, as well as define the temporal dynamic response of each stress-responsive gene. Similarly,

AU-rich elements
(AREs). Regulatory elements usually located in the 3′UTR of mRNAs that mediate recognition of an array of RNA-binding proteins and are determinant of RNA stability and translation.

Stress granules
Cytoplasmic RNA–protein complexes containing non-translating mRNAs, translation initiation components and other additional proteins that affect mRNA function. Stress granules are induced by stress and affect mRNA translation and stability.

many regulatory events have been studied and found in RNA Pol II-transcribed genes, but evidence suggests that other RNA polymerases that are dedicated to the synthesis of tRNAs, ribosomal RNAs and more are also controlled in response to stress[43].

We believe that single-cell analyses will increase in importance over the next few years, as they should provide us with a comprehensive view of basic characteristics such as the presence of thresholds and signal noise in gene expression[73] (BOX 2). The data from this type of approach will also help to determine the occupation rates and dynamics of transcription factor binding at specific promoters.

The coordinate regulation of the expression of many stress-responsive genes and the exquisite fine tuning of their expression levels might be based on the regulation of several steps in mRNA biogenesis and mRNA fate. It is conceivable that additional stress-regulatory events will be uncovered, such as at the level of nuclear structures involved in mRNA processing, mRNA export or complexes involved in cytoplasmic mRNA storage and degradation.

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FURTHER INFORMATION
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Gustav Ammerer's homepage: http://www.mfpl.ac.at/mfpl-group/group/ammerer.html

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