Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

CellPress

Trends in Biochemical Sciences

Review

Transcriptional lockdown during acute proteotoxic stress

Ritwick Sawarkar[1,*]

Cells experiencing proteotoxic stress downregulate the expression of thousands of active genes and upregulate a few stress-response genes. The strategy of downregulating gene expression has conceptual parallels with general lockdown in the global response to the coronavirus disease 2019 (COVID-19) pandemic. The mechanistic details of global transcriptional downregulation of genes, termed stress-induced transcriptional attenuation (SITA), are only beginning to emerge. The reduction in RNA and protein production during stress may spare proteostasis capacity, allowing cells to divert resources to control stress-induced damage. Given the relevance of translational downregulation in a broad variety of diseases, the role of SITA in diseases caused by proteotoxicity should be investigated in future, paving the way for potential novel therapeutics.

Lockdown: a conserved strategy during stress

When the COVID-19 pandemic mounted an unprecedented stress on the human population, one unifying theme emerged in the response of almost all governments across the world. The strategy of minimizing prepandemic human activities in some form of 'lockdown', combined with an emphasis on essential medical services, was implemented across the globe. The gene expression response of eukaryotic cells to acute proteotoxic stress has uncanny parallels to the strategy used by governments: cells downregulate thousands of genes that were active before the stress and upregulate a few hundred genes that help cells fight the damage caused by the stress.

The underlying logic of pandemic-related government response and stress-induced cellular response is likely similar. Limited resources need to be appropriately reallocated to survive the extreme conditions, diverting energy from general activities to stress-related damage control. Additionally, the continuation of general activities under stressful conditions may increase the burden on the system by contributing to the spread of the virus during the pandemic or by increasing the cellular damage during stress. The global downregulation or 'lockdown' strategy is seen at every step of gene expression: transcription, splicing, nuclear export of RNA, and translation. Global transcriptional downregulation takes place during stress alongside induction of a few stress-activated chaperone genes [1]. There is a widespread repression of splicing under stressful conditions, but stress-responsive genes continue to be spliced [2]. Nuclear transport is reduced with specialized import/export machinery in place during stress [3]. Global translation is reduced when cells experience proteotoxicity in the endoplasmic reticulum, but transcripts encoding stress-response proteins have evolved mechanisms to escape the general translational shutdown [4,5]. Reduced protein synthesis, which also occurs during other proteotoxic stress conditions, presumably spares the proteostasis machinery (e.g., chaperones and proteasomes) to focus on cellular damage caused by stress [6]. Thus, each step of gene expression applies the same general principle of activation of a few genes and downregulation of a large number of genes under conditions of proteotoxic stress.

Highlights

Acute proteotoxic stress such as heat shock causes significant transcriptional downregulation of about 50–80% of active genes along with upregulation of 2–4% genes in fly, mouse, and human genomes.

The large-scale transcriptional downregulation or stress-induced transcriptional attenuation (SITA) is not dependent on heat-shock factor (HSF), the transcription factor that is critical for upregulation of stress-response genes.

SITA is regulated at the level of RNA polymerase II elongation in mammalian cells, likely, but not exclusively during transition of promoter-proximal Pol II into elongation phase controlled by negative elongation factor (NELF).

NELF forms stress-inducible biomolecular condensates that may facilitate rapid transcriptional downregulation upon stress; the NELF condensates are similar to cytosolic stress granules in terms of stress inducibility and role in downregulation of gene expression.

[1]Medical Research Council (MRC), University of Cambridge, Cambridge, UK

*Correspondence: rs2099@cam.ac.uk (R. Sawarkar).

The molecular mechanism underlying this strategic shift requires stress sensors to be linked with the gene regulatory circuit. The pathways uncovered so far have not only contributed to our understanding of fundamental cell biology but have also led to therapeutics [7]. This review will focus on the 'lockdown' strategy applied by stressed cells at the first step of gene expression, namely transcription.

SITA

The transcriptional response to proteotoxic stress was uncovered in a serendipitous observation by Ritossa in the 1960s [8]. Heat-shock puffs observed on drosophila (*Drosophila melanogaster*) salivary gland polytene chromosomes represented the induction of chaperones such as HSP70 and HSP90 [9,10]. The rapid upregulation of chaperone genes has been extensively studied in the past two decades, providing insightful mechanisms of stress sensing and transcriptional response [11]. Chaperone induction upon stress is coordinated by a family of **heat-shock factors (HSFs**) (see Glossary) [12].

Early studies of radioactive labeling of nascent transcripts on drosophila polytene chromosomes had also suggested that chaperone upregulation is accompanied by general transcriptional downregulation of nonchaperone genes (Figure 1A,B) [13,14]. Recent work using state-of-the-art genome-wide technologies to quantify nascent transcription confirmed the global transcriptional downregulation during stress (Figure 1C) [1,15–18]. We termed the cellular response leading to the global transcriptional downregulation as **stress-induced transcriptional attenuation (SITA)** [16]. In independent studies using different techniques and distinct cell types of fly, mouse, and human origin, a consistent picture has emerged: heat shock causes significant transcriptional downregulation of about 50–80% of active genes, along with upregulation of 2–4% of genes in the genome [1,15–17]. The large majority of downregulated genes, or SITA target genes, are highly active in cells before stress exposure, and typically encode proteins responsible for metabolism, the cell cycle, and protein biosynthesis [1]. A kinetic analysis of gene expression changes has shown that 20–25% of active genes are downregulated within 12 min of exposure to heat shock, with the proportion of downregulated genes reaching 50–80% within an hour [1]. Importantly, SITA is independent of HSF as shown by studies on cells deficient in HSF1 and HSF2 [1,16,17], suggesting that SITA represents a novel branch of transcriptional response to stress. Thus, an acute proteotoxic stress such as heat shock causes a rapid and global transcriptional downregulation. Conceptually, the process of global downregulation is similar to the repressive environmental stress response in yeast, although the mechanism may be different in yeast and mammalian cells [19]. Remarkably, cells show a similar global downregulation of transcription under conditions of oxidative stress and DNA damage [20,21]. The overlap between genes downregulated by different stresses has not been studied, but it is likely that SITA under various stress conditions shares common principles and mechanisms.

While numerous studies have identified intricate molecular details of chaperone induction, we know very little about the mechanistic basis of SITA. Due to steady-state levels of pre-existing RNA during stress, SITA is seen only in assays quantifying nascent transcription [22]. Stress-mediated induction of chaperone genes is easily studied by simple qPCR assays, facilitating mechanistic studies of HSF-mediated upregulation, unlike SITA. Several basic questions need to be answered. What is the molecular mechanism underlying SITA? What is the functional significance of SITA, if any? What is the relationship between SITA and HSF-mediated chaperone induction, given that both processes take place simultaneously in the same nucleus? This review critically summarizes the current state of our understanding of these questions and charts future avenues of investigation.

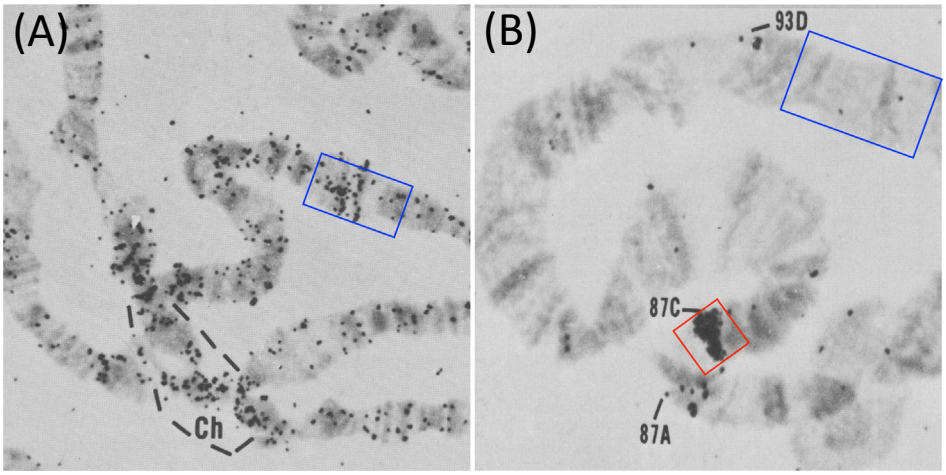
Glossary

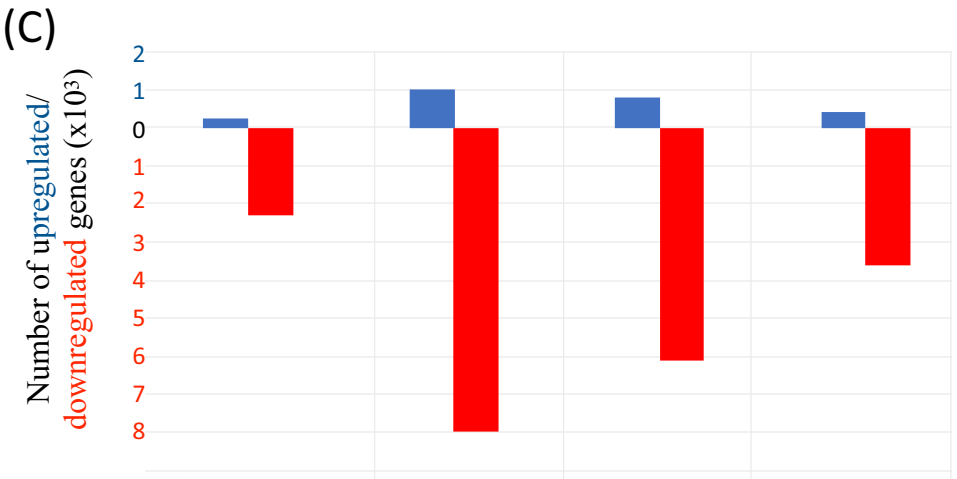
Heat-shock factors (HSFs): transcription factors that are activated by various cellular stresses; they bind heat-shock elements near stress response genes, such as heat-shock proteins (e.g., Hsp70).

Intrinsically disordered regions (IDRs): polypeptide stretches within proteins that have low sequence complexity and do not form known structures under experimental conditions tested.

Liquid–liquid phase separation: the process by which a protein solution de-mixes into protein-dense and protein-light phases. This process is likely critical for the formation of membraneless organelles within cells.

Stress-induced transcriptional attenuation (SITA): the process by which stressed cells downregulate a large proportion of active genes.





Cells	Drosophila (S2)	Mouse (MEF)	Human (K562)	Human (HEK293T)
Technique	PRO-seq	PRO-seq	PRO-seq	Pol II ChIP-seq
Stress	20 min at 37°C	60 min at 42°C	30 min at 42°C	90 min at 43°C
Source	Duarte et al. [17]	Mahat et al. [1]	Vihervaara et al. [15]	Aprile-Garcia et al. [16]

Trends in Biochemical Sciences

Figure 1. Global transcriptional changes caused by heat shock in metazoan. (A,B) Autoradiographs of drosophila salivary polytene chromosomes after exposure to normal (A) or heat-shock (B) temperatures. The chromosomes were treated to perform *in situ* hybridization with H^3-labeled mRNA. The red box indicates the position of the 'heat-shock puff' representing chaperone HSP70 induction. The blue boxes indicate arms of chromosomes with transcription under normal conditions that is downregulated during heat shock. Panels (A) and (B) reproduced, with permission, from Spradling *et al.* [14]. (C) A summary histogram showing the number of upregulated and downregulated genes in heat-shocked cells in four independent studies. Cell type used in the study, stress regimes, and techniques used to assay nascent transcription in each study are indicated in the table below the histogram. Abbreviations: PRO-seq, precision run-on sequencing. See also [1,15–17].

Stress sensors for SITA

How do cells sense stress and communicate the information to transcriptional effectors in order to cause SITA? An approach of small-throughput chemical screening identified three cellular processes which, when inhibited, block SITA: nascent translation, protein ubiquitination, and

p38 kinase [16]. Follow-up studies based on chemical screening led to a working model that the protein ubiquitination during heat shock activates p38 kinase, which then translocates into the nucleus to drive SITA by directly binding to target promoters [16]. One of the first events leading to SITA is the ubiquitination of nascent polypeptides when cells are exposed to stress. p38 kinase activation and nuclear translocation during stress requires protein synthesis and ubiquitination. ChIP-seq of the kinase showed that p38 binds to promoters of SITA target genes, and its kinase activity is required for SITA [16].

It is thought that polypeptides emerging out of the ribosome may be exquisitely sensitive to proteotoxic stress, and likely misfold as the chaperone capacity of the cell is compromised in the first few minutes of acute proteotoxic stress [23]. The link between nascent translation and SITA likely may reflect a strategy of cells to quantitatively calibrate their transcriptional response to the translational flux of that cell normalized to their chaperoning capacity. Cells with a higher translation rate or lower chaperone capacity may show higher nascent protein ubiquitination, likely providing stronger signal to drive SITA. A quantitative comparison between translation flux, stress-induced ubiquitination on nascent polypeptides, and extent of transcriptional downregulation may provide direct evidence for a quantitative relationship between stress sensor and SITA. The link between cytosolic translation and nuclear transcription in a stressed cell may simply be mediated by a short-lived transcriptional regulatory protein such as Myc [24,25]. Blocking translation with cycloheximide in the absence of stress does not lead to global downregulation of transcription [16], ruling out the involvement of a short-lived mediator of transcription–translation communication.

While circumstantial evidence available thus far does not negate the ubiquitination–p38–SITA pathway, several questions remain to be answered. Which ubiquitin ligase(s) is/are involved in nascent polypeptide ubiquitination upon stress? How does p38 kinase get activated specifically upon ubiquitination of nascent polypeptides? How does p38 kinase translocate to the nucleus, bind SITA-target promoters, and drive SITA? Are lipid-membrane- or RNA-based stress sensors involved in SITA? Could there be a reallocation of ubiquitin diverted from histones to nascent polypeptides during stress, leading to direct physical changes in chromatin accessibility? Answers to these questions will not be straightforward owing to the pleiotropic nature of the involved proteins and pathways. Careful dissection of the cause–consequence relationship between different pathways will be an area for future investigation. Furthermore, it would be interesting to see whether and how stresses beyond acute proteotoxic heat shock implement a similar stress sensory circuit in order to drive SITA. Oxidative stress, nutrient deprivation, and mTOR signaling will be exciting avenues to study SITA in a physiological context.

The transcriptional effectors of SITA

How is transcriptional downregulation of SITA-target genes achieved at a molecular level? RNA polymerase II (Pol II) activity is regulated at several steps during transcription (Figure 2A) [26–30]. Genome-wide assessment of transcription is done using various techniques such as precision run-on sequencing (PRO-seq) and Pol II ChIP-seq [22]. Recent studies using these techniques have shown that the levels of promoter-proximal Pol II at downregulated genes do not decrease upon heat-shock stress, but transcription through the body of the target genes decreases [1,15,16]. These data suggest that heat shock affects Pol II during the transition to the transcriptional elongation phase. Specifically, stress likely leads to an increase in the stability or duration of promoter-proximally paused Pol II; in other words, Pol II pauses downstream of promoters for a much longer time during stress. However, the current genome-wide data do not rule out other molecular models explaining the effect of stress on Pol II dynamics [30,31]. In the absence of stress, promoter-proximal Pol II often undergoes premature termination instead

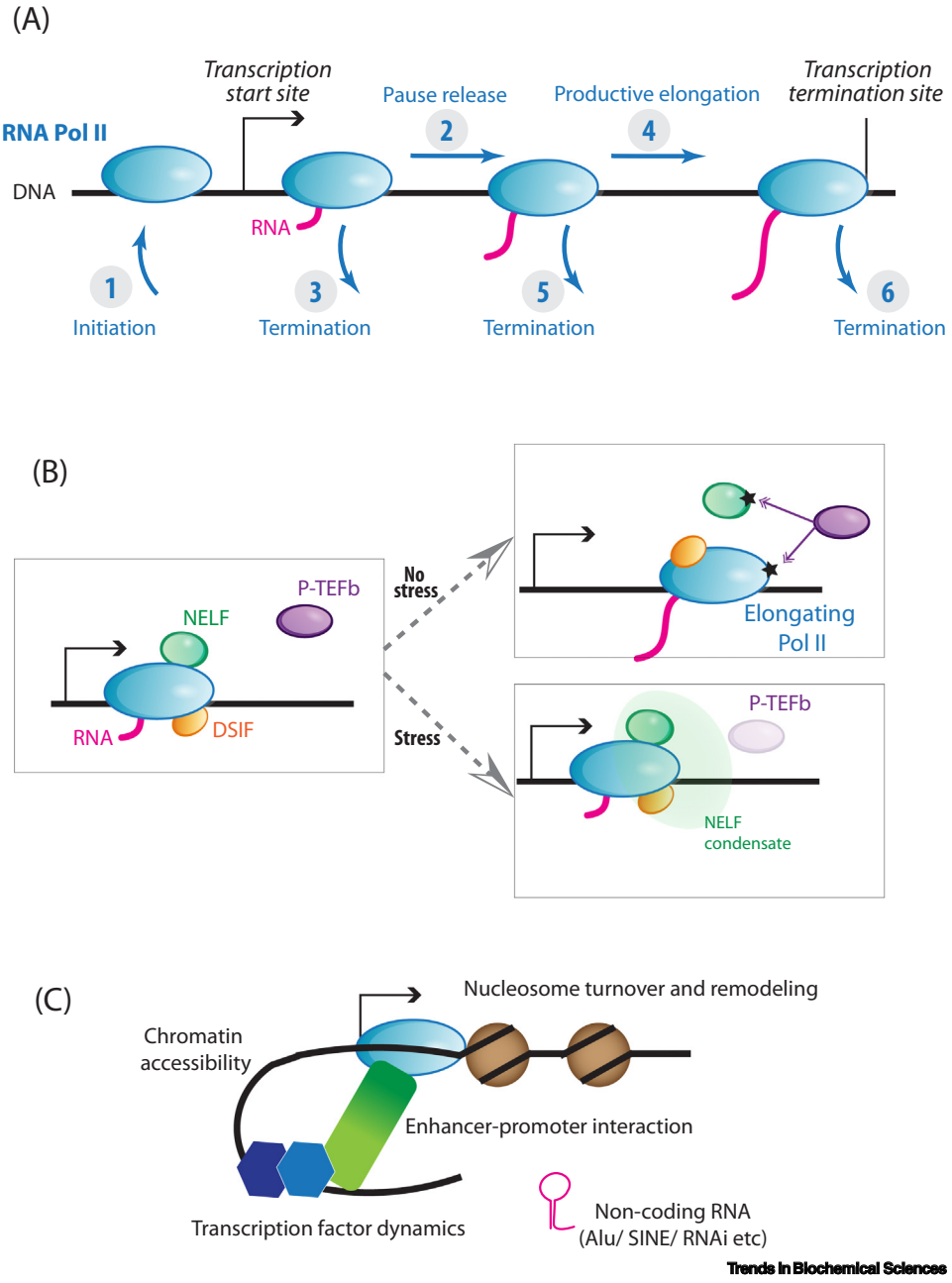


Figure 2. Molecular mechanisms underlying stress-induced transcriptional attenuation. (A) A schematic illustration showing various steps during mRNA transcription by Pol II that are subject to potential regulation by stress. The steps are numbered in the schematic as follows: initiation and RNA Pol II complex assembly (1), promoter-proximal Pol II subject to transition to elongation (2) or release from chromatin (3), elongating Pol II subject to regulation of speed (4) and premature termination (5), and termination of Pol II after full mRNA transcription (6). (B) The current understanding of the likely regulation of Pol II at stress-induced transcriptional attenuation (SITA) target genes under unstressed and stressed conditions. The black stars denote phosphorylation mediated by positive transcription elongator factor b (P-TEFb). Stress leads to a decrease in the active P-TEFb pool. (C) Additional regulatory layers of transcription in the context of chromatin that may be affected by stress. Abbreviations: NELF, negative elongation factor; DSIF, DRB-sensitivity-inducing factor.

of escaping into productive elongation. Stress might block premature termination to stabilize Pol II near promoters. Another possibility is that stress affects the speed of Pol II elongation, or causes premature termination at various points along the length of the gene [32]. A recent study lends support to this possibility of termination along the gene body [33]. Experiments that quantify the Pol II dynamics, elongation speeds, and termination frequency [34,35] are required to identify how different steps in the transcription cycle could be affected during stress.

How might stress affect promoter-associated Pol II? The transition of promoter-proximal Pol II into elongating Pol II is regulated by several factors in a metazoan cell, highlighting the importance of this transition in gene expression (Figure 2B) [27]. An interplay between positive and negative regulators of transcriptional elongation decides the fate of promoter-proximal Pol II. Complexes such as the negative elongation factor (NELF) and 5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole (DRB) sensitivity-inducing factor (DSIF) oppose the transition of Pol II in the elongating form [26]. Recent structural studies have revealed that NELF restrains Pol II mobility by contacting the trigger loop [36]. In addition, NELF prevents binding of the transcription elongation factor IIS (TFIIS) to Pol II. NELF is also required by Pol II to effectively overcome the nucleosomal barrier and to enter productive elongation [37,38]. Positive transcription elongator factor b (P-TEFb), consisting of CDK9 and cyclin T, phosphorylates NELF, DSIF, and Pol II to release NELF from chromatin, facilitating the transition of promoter-proximal Pol II in the gene body [39]. Heat-shock stress likely acts on multiple players in this circuit to affect the release of promoter-proximal Pol II in gene bodies.

Chromatin proteomics and ChIP-seq data have shown that the amount of NELF and DSIF associated with chromatin and SITA target promoters dramatically increases upon heat shock, providing a stronger negative force to stop transition to elongation (Figure 2B) [16]. Simultaneously, stress causes an increase in interaction between P-TEFb and its negative regulator the HEXIM complex, likely leading to a depletion in the levels of active P-TEFb (Figure 2B) [40]. The end result of increased chromatin-associated NELF and reduced active P-TEFb is a substantial decrease in the levels of elongating Pol II leading to rapid transcriptional downregulation of SITA target genes. It is not known whether there is a coordination between increased NELF at chromatin and depletion of active P-TEFb; it is likely that both these events are independently regulated by stress. It is also not clear whether and how RNA pol II activity is regulated at stages other than transition of Pol II to elongation stage.

Target specificity of SITA in the context of chaperone induction

The simple model involving inactivation of P-TEFb and increased chromatin recruitment of NELF poses several contradictions. P-TEFb activity is essential for HSF-mediated induction of chaperone genes [41]. Even if stress leads to binding of P-TEFb to inactivating the HEXIM complex, there may be a pool of active P-TEFb accessible to HSF to induce chaperone genes. It is not clear why such a pool specifically works with HSF but not with other transcription factors that regulate SITA target genes. Detailed biochemical experiments will be required to resolve this contradiction which is at the heart of the target specificity of SITA.

The second contradiction posed by the model described earlier is the observation that NELF is also required for HSF-mediated induction of chaperone genes [42]. NELF abundance increases at promoters of downregulated genes, but its levels at promoters of upregulated chaperone genes are also increased upon stress [16]. A likely explanation for this contradiction is that there is an increased recruitment of Pol II at chaperone promoters, effectively keeping the ratio of NELF to Pol II constant at these genes. However, at SITA target genes there is no increase in Pol II, effectively increasing NELF concentration with respect to Pol II, leading to downregulation [40].

A third contradiction is the observation that even some positive elongation factors (e.g., PAF1) are shown to increase at SITA target promoters [16]. Classically shown to be a positively acting factor, PAF1 has recently been shown to oppose Pol II elongation, thus effectively acting alongside NELF [43]. Recent structural evidence, however, shows that NELF and PAF cannot simultaneously bind to Pol II [36,44]. This contradiction needs to be resolved by combining *in vitro* transcription assays using heat-shocked nuclear extracts and structure–function experiments.

Finally, the model does not show how a subset of genes, such as chaperone genes, escape global downregulation. Two experiments might provide a partial explanation of this conundrum. First, in HSF knockout cells, chaperone genes are not induced as expected, but in fact they are transcriptionally downregulated upon stress [45]. Second, by comparing the heat-shock response in the presence and absence of fresh serum, it is clear that stress downregulates serum-response factor (SRF)-driven genes in the absence of serum, but these genes escape downregulation in the presence of serum [1,45]. Both of these observations suggest that transcriptional downregulation is a global event that does not show any specificity. A small subset of activated transcription factors such as HSF and SRF may locally oppose the transcriptional downregulation, allowing their target genes to be upregulated during stress. Which biochemical properties of these transcription factors are responsible for their ability to oppose global down-regulation will be an important question for future research.

NELF condensation and potential nuclear counterparts of cytosolic stress granules

How does stress lead to enhanced NELF recruitment at chromatin? A recent study reported an interesting observation that NELF rapidly reorganizes into biomolecular condensates in the nucleus upon exposure to stress, likely by phase separation (Figure 3) [40]. The process of

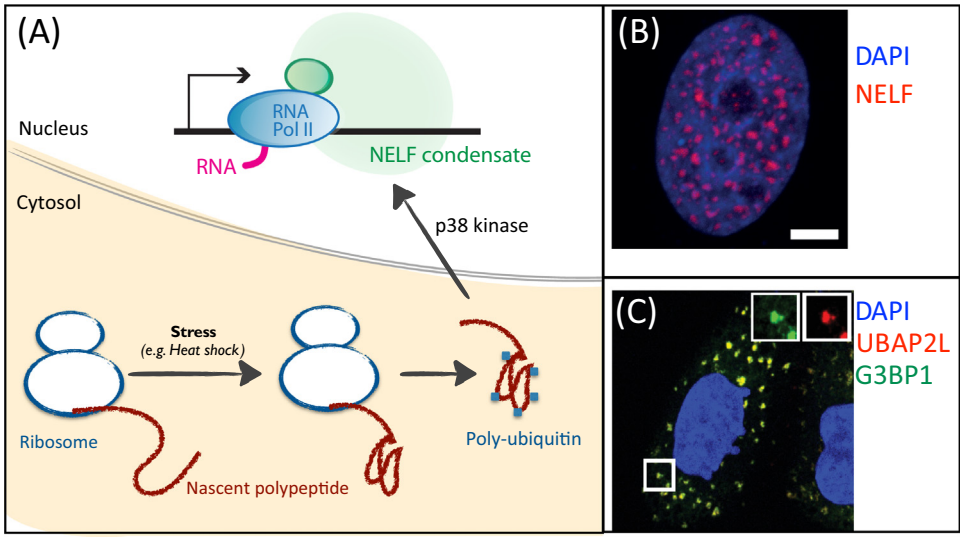


Figure 3. Stress-induced signaling and dynamic compartmentalization in the nucleus and the cytosol. (A) A schematic illustration showing our current understanding of stress signaling leading to stress-induced transcriptional attenuation (SITA). Nascent polypeptides on the ribosome are subject to polyubiquitination, activating p38 kinase and its translocation to the promoters of downregulated genes. Polyubiquitin denotes a chain of ubiquitin proteins added to the nascent polypeptide. (B,C) Confocal images showing nuclear negative elongation factor (NELF) condensates (B) and cytosolic stress granules (C) formed as a result of heat shock in HeLa cells. Markers of the nuclear and cytosolic granules are indicated. Abbreviation: DAPI, 4′,6-diamidino-2-phenylindole. (B) Reproduced, with permission, from Rawat *et al*. [40] and (C) reproduced, with permission, from Cirillo *et al*. [53].

condensation and **liquid–liquid phase separation** has recently emerged as a nonclassical way of organization of cellular processes, including transcription [28,46]. NELF condensates represent one of the few stress-inducible condensates, such as nuclear stress bodies occupied by HSF [47] and others formed by noncoding RNA [48].

While it is not clear whether NELF condensates form at or near SITA target promoters, *in vitro* studies show that NELF condensates enrich synthetic peptides with the Pol II C-terminal domain sequence phosphorylated at serine-5 [40]. The serine-5-phosphorylated form of Pol II is present mostly at promoter-proximal regions [26] where stress causes an increased NELF recruitment, suggesting that NELF condensates may form at SITA target promoters in stressed cells. NELF harbors two **intrinsically disordered regions** (**IDRs**), one in each of its constituent proteins (NELFA and NELFE). A deletion of NELFA IDR blocked NELF condensation upon stress, providing a genetic handle to address the role of NELF condensation to SITA [40]. Unexpectedly, NELFA IDR mutant bound normally to target promoters but did not increase at promoters upon stress. Importantly, cells expressing NELFA IDR deletion mutant did not downregulate SITA target genes, although chaperone induction was near normal. These observations suggest that NELF condensation is required for stress-enhanced retention of NELF at SITA target promoters. Cells expressing NELFA-IDR deletion mutants with defective SITA were found to be stress-sensitive [40], providing important evidence that SITA is critical for cellular survival upon stress. Thus, rapid and dynamic compartmentalization of nuclear processes may present a molecular model of how stress induces global transcriptional changes.

Microscopically visible condensates of NELF formed upon stress may suggest that SITA target genes cluster together by changes in chromatin conformation during stress. Assays to measure changes in 3D conformation of chromosomes have been used to study whether global transcriptional reprogramming upon stress is accompanied by local or global conformation changes. However, the results from different studies do not agree with each other. Chromatin conformation was found to remain stable during heat shock in human K562 cells and drosophila S2 cells [49]. In independent studies, widespread rearrangement of 3D chromatin reorganization was seen in heat-shocked drosophila Kc167 cells and human H9 embryonic stem cells [50,51]. It remains to be seen whether the contrasting results are due to different cell types or to distinct technical methods employed.

Given their stress inducibility and their important role in transcriptional downregulation, NELF condensates could represent potential nuclear counterparts of well-known cytosolic stress granules (Figure 3C). Research over the past few years has shown that stress granules play an important role in downregulating translation in stressed cells [52]. Moreover, NELF condensates are formed during the same stressful conditions as the cytosolic stress granules, and with similar kinetics. It is noteworthy that the primary initiating stimulus for SITA is nascent protein ubiquitination and translation [16]. Protein ubiquitination and ribosomes are associated with cytosolic stress granules which are marked by proteins such as ubiquitin-associated protein 2 like (UBAP2L) and GAP SH3 domain-binding protein 1 (G3BP1) (Figure 3C) [53]. It will be exciting to see whether there is a direct communication between cytosolic stress granules and nuclear NELF condensates. While NELF is not conserved in all metazoa, SPT5 – a member of the DSIF complex – may perform the function of NELF in organisms such as *Caenorhabditis elegans* that lack NELF. Indeed, human SPT5 protein is a part of the NELF condensates formed upon stress [40].

How does stress lead to NELF condensation? Stress such as heat shock activates a specific SUMO (small ubiquitin-like modifier) E3 ligase called ZNF451 that modifies the NELF complex with poly-SUMOylation on several sites [40]. Interestingly, the NELF complex harbors at least

one strong SUMO interaction motif (SIM). It is likely that poly-SUMOylation on the NELF complex provides multivalency for interaction between NELF SIM, thus causing an increased local concentration of NELF which seeds condensation driven by its IDRs. P-TEFb-mediated phosphorylation of NELF is shown to antagonize NELF condensation. Thus two independent events – namely P-TEFb inactivation and ZNF451 activation – have to occur simultaneously to cause NELF condensation. The necessity for two independent changes in post-translational modifications leading to NELF condensation may reflect a cellular strategy of committing to SITA only when there is a high level of stress.

The broader transcriptional context of SITA target genes

In addition to the promoter-centric control, the process of transcriptional downregulation may also be influenced by enhancer–promoter interactions which may be sensitive to stress (Figure 2C) [54]. Enhancers, among several distal regulatory elements, have been shown to be transcriptionally upregulated by heat shock, when their cognate promoters presumably show decreased expression [15]. Binding of a number of transcription factors and chromatin remodelers to SITA targets is shown to change during heat shock (Figure 2C) [51]. Consequently, there may be critical changes in chromatin accessibility at SITA targets upon stress. Studies in drosophila have shown that there is a significant reduction in nucleosome turnover or mobility in gene bodies of SITA target genes [18]. Whether these changes are a cause or a consequence of transcriptional downregulation will be an important question to be addressed in the near future. There is also an increased SUMOylation of chromatin proteins upon acute proteotoxic stress such as heat shock, mainly by conjugation of SUMO-2 to a number of Pol II regulatory proteins [55–57]. SUMOylation may be critical to stabilize the chromatin-regulatory complexes during stress, and may indirectly regulate transcription, chromatin conformation, promoter–enhancer interaction, etc. 'Group SUMOylation' of a number of residues on protein complexes, rather than specific residues on a few proteins, is thought to occur on chromatin upon stress [56]. The comprehensive identification of enzymes responsible for chromatin SUMOylation and how these SUMO ligases are activated by stress are not fully known.

While mammalian cells retain promoter-proximal binding of Pol II upon heat-shock stress, drosophila cells significantly lose Pol II from promoters of SITA target genes during heat shock [17,18]. These results suggest an additional regulatory layer at transcription initiation in flies subjected to higher temperatures (Figure 2A). The divergent mechanisms in flies versus mammalian cells may be due to the fact that flies do not regulate their body temperature and are more often subject to temperature changes unlinked to proteotoxic stress. Nonetheless, careful studies have shown that the subnucleosomal pattern at drosophila promoters is retained during stress [18,58]. An interpretation of these findings is that transcription factors and associated chromatin regulators may be retained at SITA target genes during stress, even if Pol II is lost from promoter-proximal sites (Figure 2C).

Remarkably, RNA interference (RNAi) machinery has been shown to play a role in loss of Pol II from SITA target promoters in drosophila, likely by interacting with the NELF complex [59]. A subunit of the NELF complex, NELFE, has a putative RNA-recognition motif [60] which may bind to small noncoding RNA produced by RNAi machinery. Whether RNAi plays a role in mammalian SITA or not has not been investigated. Other noncoding RNAs – such as SINE and Alu elements – are shown to disrupt the interaction between Pol II and DNA (Figure 2C), specifically by preventing Pol II from forming the closed complex, the first step in transcription [61,62]. Thus, SINE/Alu–Pol II complex may remain at promoters bound to TATA-box-binding protein (TBP), but is unable to enter into a productive transcription cycle, leading to transcriptional repression. Both SINE and Alu RNAs are induced during recovery after cells are withdrawn from heat shock [63]. It is not clear whether SINE/Alu elements play any role in SITA which is

initiated within minutes of heat shock, when the levels of SINE/Alu are similar to those in unstressed cells. However, SINE/Alu may play a role in maintaining transcriptional downregulation once established in the early phase of stress response. The role of noncoding RNAs in SITA and the divergence between flies and mammalian cells will be insightful areas for future research.

SITA and global translational repression

Stressed cells downregulate gene expression at all levels, including mRNA translation on the ribosome, as well as global transcriptional downregulation in the form of SITA. A number of environment-responsive protein kinases phosphorylate eIF2α, leading to a block in translation initiation [64]. Such a response of global translational downregulation nonetheless allows a subset of mRNA to be translated by making use of an upstream open reading frame (ORF) within the mRNA [5]. Thus, global downregulation and specific upregulation take place both at translational and transcriptional levels at the same time in the same cell. An exciting avenue to investigate is a potential mechanistic link or feedback between SITA and translational downregulation. There may be a common upstream regulator that initiates downregulation at both transcriptional and translational levels. Another possibility is that stress directly causes downregulation at the translational level, and SITA is an indirect consequence of translational downregulation. The latter model may also work in the opposite way, in that stress directly causes SITA, and translational downregulation is a consequence of the decrease in mRNA synthesis. Whether and how translational and transcriptional downregulation are coordinated with a block in splicing and nuclear/cytoplasmic transport during stress needs to be further investigated.

Both SITA and translational downregulation likely provide a survival benefit to stressed cells, possibly by sparing proteostasis capacity due to downregulated transcription and translation, respectively [7,16,40]. Neurodegeneration, cancer, and aging represent human conditions in which cells show signs of proteotoxic damage [65–67]. Whereas translational downregulation has been shown to occur in many human pathologies [68], it remains unknown whether SITA is also seen in these cases and whether it plays any role in pathogenesis. Nonetheless, therapeutic targeting of translational downregulation by activating or inhibiting the pathway has been shown to provide benefit in different circumstances [68–70]. In particular, small-molecule regulators of eIF2α phosphorylation have proved to beneficially alter translation downregulation in animal models of proteotoxic diseases. Continued downregulation of translation may sometimes favor cellular survival, depending on the phase of the disease. It will be interesting to see whether manipulation of transcriptional downregulation may provide a new opportunity for disease treatment, especially in those cases where targeting translational downregulation has already proved beneficial [68]. Genetic models that disrupt SITA may provide the proof-of-concept that targeting SITA is of therapeutic importance. A simultaneous targeting of transcriptional and translational downregulation may prove to be synergistic as effective repression of gene expression may be achieved. Independently of the likely contribution of SITA to restoring proteostasis capacity, reduced transcription may also safeguard the genome against potential DNA damage during stress. Thus, a comprehensive understanding of the molecular basis of SITA may suggest novel avenues for therapeutic intervention. Small molecules targeting the p38–CDK9–NELF axis may prove useful; however, the broad physiological roles played by these proteins may necessitate more specific methods of therapeutic intervention.

Concluding remarks

Acute proteotoxic stress such as heat shock induces rapid transcriptional changes in the form of global downregulation (SITA) and upregulation of a few chaperone genes. Both of these events have to occur in the same nucleus at the same time. It is not clear whether SITA and chaperone upregulation are coordinated with each other in space and time, even though SITA occurs in the

Outstanding questions

Stress causes transcriptional downregulation of a large number of active genes, termed SITA. How are the stress sensors connected at a molecular level to transcriptional effectors of SITA?

What are the mechanisms by which RNA pol II activity is reduced on active genes while induction of chaperone genes is still allowed? What are the roles of chromatin, noncoding RNA, and chromosome conformation in the stress response?

Is there coordination between translational and transcriptional downregulation that occurs in the same cell at the same time under conditions of stress?

Does physiological and pathological proteotoxic stress also lead to SITA *in vivo*? If so, would alterations of SITA help in disease therapeutics?

absence of chaperone induction in HSF-deficient cells [1,16]. Single-cell-based assays such as simultaneous visualization of loci with upregulated and downregulated genes in living cells will be required to study coordination between SITA and chaperone upregulation. It is interesting to note that nuclear stress bodies observed as HSF puncta in stressed nuclei [12,71,72] do not overlap with NELF condensates [40], suggesting spatial segregation of chaperone upregulation and SITA. Whether or not heat shock causes global changes in chromosome conformation remains controversial, and high-resolution conformation dynamics need to be studied to understand the spatial coordination between chaperone upregulation and SITA. An investigation into temporal coordination between transcriptional upregulation and downregulation also requires single-cell nascent transcriptomics. Does SITA occur gradually with downregulation of some genes on high priority? Are chaperone genes sequentially activated along with downregulation of specific sets of genes? Single-cell resolution of nascent transcriptome using recently developed techniques [73] will provide answers to these questions. It will be important to identify the mechanisms underlying spatial and temporal coordination between transcriptional upregulation and downregulation, if any (see Outstanding questions).

How do cells reset their transcriptional program during recovery from stress? Is there a sequence of events that recapitulates the onset of the response in a reverse order? Does chaperone expression persist for a longer time compared with SITA during recovery? It is noteworthy that NELF condensates take about an hour of recovery from heat shock to completely disappear [40], in line with the observed recovery of SITA [74]. Not all nuclear compartments recover after stress [75], suggesting distinct mechanisms operating on different nuclear compartments. Chaperones may play a role in the process of dissolving NELF condensates during recovery as a negative feedback mechanism [76], likely linking the chaperone capacity to the decision of resetting the transcriptional landscape to normality. Protein levels of many chaperones continue to be elevated after stress, allowing chaperone-mediated dissolution of NELF condensates. Chromatin modifications and remodeling accompany transcriptional changes during acute heat shock, but how are they reset during recovery? Recent work suggests that cells which were repeatedly stressed continued downregulating SITA targets long after recovery from stress [74]. This memory of SITA after repeated exposure to stress persists through several mitotic events. How is this memory of stress encoded in the cell? Stress may alter chromatin modifications that may persist through mitoses, or specific RNAs/proteins made during stress may persist for a long time, providing memory *in trans*. In this regard, it is noteworthy that SINE/Alu elements are induced during recovery from heat shock [61]. These Pol III-transcribed short noncoding RNAs have been shown to repress Pol II activity [62], and may themselves represent agents for memory.

Global translational downregulation that accompanies SITA in stressed cells has turned out to be an attractive therapeutic target, especially in protein misfolding diseases [68]. A careful dissection of mechanisms underlying SITA will allow us to test whether targeting SITA in misfolding diseases could also be beneficial in treatment. The prevalence of SITA in pathologies beyond protein misfolding will be an exciting avenue for future investigations likely leading to novel therapeutics.

Acknowledgments

I would like to thank the past and present members of my laboratory for scientific discussions, and anonymous reviewers for their critical feedback on the review. Research in my laboratory is supported by the Medical Research Council (UK), the University of Cambridge, the German Research Foundation (DFG) under Germany's Excellence Strategy – EXC-2189 – Project ID 390939984, and a European Research Council Consolidator grant (819753).

Declaration of interests

No interests are declared.

References

1. Mahat, D.B. *et al.* (2016) Mammalian heat shock response and mechanisms underlying its genome-wide transcriptional regulation. *Mol. Cell* 62, 63–78
2. Shalgi, R. *et al.* (2014) Widespread inhibition of posttranscriptional splicing shapes the cellular transcriptome following heat shock. *Cell Rep.* 7, 1362–1370
3. Kose, S. *et al.* (2012) Hikeshi, a nuclear import carrier for Hsp70s, protects cells from heat shock-induced nuclear damage. *Cell* 149, 578–589
4. Lu, P.D. *et al.* (2004) Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response. *J. Cell Biol.* 167, 27–33
5. Harding, H.P. *et al.* (2000) Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol. Cell* 6, 1099–1108
6. Harding, H.P. *et al.* (2000) Perk is essential for translational regulation and cell survival during the unfolded protein response. *Mol. Cell* 5, 897–904
7. Luh, L.M. and Bertolotti, A. (2020) Potential benefit of manipulating protein quality control systems in neurodegenerative diseases. *Curr. Opin. Neurobiol.* 61, 125–132
8. Ritossa, F. (1962) A new puffing pattern induced by temperature shock and DNP in Drosophila. *Experimentia* 18, 571–573
9. Lindquist, S. (1986) The heat-shock response. *Annu. Rev. Biochem.* 55, 1151–1191
10. Tissières, A. *et al.* (1974) Protein synthesis in salivary glands of *Drosophila melanogaster*: relation to chromosome puffs. *J. Mol. Biol.* 84, 389–398
11. Li, J. *et al.* (2017) Rethinking HSF1 in stress, development, and organismal health. *Trends Cell Biol.* 27, 895–905
12. Akerfelt, M. *et al.* (2010) Heat shock factors: integrators of cell stress, development and lifespan. *Nat. Rev. Mol. Cell Biol.* 11, 545–555
13. Jamrich, M. *et al.* (1977) Localization of RNA polymerase in polytene chromosomes of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U. S. A.* 74, 2079–2083
14. Spradling, A. *et al.* (1975) Analysis of drosophila mRNA by in situ hybridization: sequences transcribed in normal and heat shocked cultured cells. *Cell* 4, 395–404
15. Vihervaara, A. *et al.* (2017) Transcriptional response to stress is pre-wired by promoter and enhancer architecture. *Nat. Commun.* 8, 255
16. Aprile-Garcia, F. *et al.* (2019) Nascent-protein ubiquitination is required for heat shock-induced gene downregulation in human cells. *Nat. Struct. Mol. Biol.* 26, 137–146
17. Duarte, F.M. *et al.* (2016) Transcription factors GAF and HSF act at distinct regulatory steps to modulate stress-induced gene activation. *Genes Dev.* 30, 1731–1746
18. Teves, S.S. and Henikoff, S. (2011) Heat shock reduces stalled RNA polymerase II and nucleosome turnover genome-wide. *Genes Dev.* 25, 2387–2397
19. Ho, Y.H. and Gasch, A.P. (2015) Exploiting the yeast stress-activated signaling network to inform on stress biology and disease signaling. *Curr. Genet.* 61, 503–511
20. Machour, F.E. and Ayoub, N. (2020) Transcriptional regulation at DSBs: mechanisms and consequences. *Trends Genet.* 36, 981–997
21. Nilson, K.A. *et al.* (2017) Oxidative stress rapidly stabilizes promoter-proximal paused Pol II across the human genome. *Nucleic Acids Res.* 45, 11088–11105
22. Wissink, E.M. *et al.* (2019) Nascent RNA analyses: tracking transcription and its regulation. *Nat. Rev. Genet.* 20, 705–723
23. Xu, G. *et al.* (2016) Vulnerability of newly synthesized proteins to proteostasis stress. *J. Cell Sci.* 129, 1892–1901
24. Lin, C.Y. *et al.* (2012) Transcriptional amplification in tumor cells with elevated c-Myc. *Cell* 151, 56–67
25. Hann, S.R. and Eisenman, R.N. (1984) Proteins encoded by the human c-myc oncogene: differential expression in neoplastic cells. *Mol. Cell. Biol.* 4, 2486–2497
26. Dollinger, R. and Gilmour, D.S. (2021) Regulation of promoter proximal pausing of RNA polymerase II in metazoans. *J. Mol. Biol.* 433, 166897
27. Adelman, K. and Lis, J.T. (2012) Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. *Nat. Rev. Genet.* 13, 720–731
28. Palacio, M. and Taatjes, D.J. (2021) Merging established mechanisms with new insights: condensates, hubs, and the regulation of RNA polymerase II transcription. *J. Mol. Biol.* 434, 167216
29. Noe Gonzalez, M. *et al.* (2021) Causes and consequences of RNA polymerase II stalling during transcript elongation. *Nat. Rev. Mol. Cell Biol.* 22, 3–21
30. Ehrensberger, A.H. *et al.* (2013) Mechanistic interpretation of promoter-proximal peaks and RNAPII density maps. *Cell* 154, 713–715
31. Vihervaara, A. *et al.* (2018) Molecular mechanisms driving transcriptional stress responses. *Nat. Rev. Genet.* 19, 385–397
32. Kamieniarz-Gdula, K. and Proudfoot, N.J. (2019) Transcriptional control by premature termination: a forgotten mechanism. *Trends Genet.* 35, 553–564
33. Cugusi, S. *et al.* (2022) Heat shock induces premature transcript termination and reconfigures the human transcriptome. *Mol. Cell.* https://doi.org/10.1016/j.molcel.2022.01.007. S1097-2765(22)00007-7
34. Krebs, A.R. *et al.* (2017) Genome-wide single-molecule footprinting reveals high RNA polymerase II turnover at paused promoters. *Mol. Cell* 67, 411–422.e414
35. Steurer, B. *et al.* (2018) Live-cell analysis of endogenous GFP-RPB1 uncovers rapid turnover of initiating and promoter-paused RNA Polymerase II. *Proc. Natl. Acad. Sci. U. S. A.* 115, E4368–E4376
36. Vos, S.M. *et al.* (2018) Structure of paused transcription complex Pol II-DSIF-NELF. *Nature* 560, 601–606
37. Aoi, Y. *et al.* (2020) NELF regulates a promoter-proximal step distinct from RNA Pol II pause-release. *Mol. Cell* 78, 261–274.e265
38. Core, L.J. *et al.* (2012) Defining the status of RNA polymerase at promoters. *Cell Rep.* 2, 1025–1035
39. Zhou, Q. *et al.* (2012) RNA polymerase II elongation control. *Annu. Rev. Biochem.* 81, 119–143
40. Rawat, P. *et al.* (2021) Stress-induced nuclear condensation of NELF drives transcriptional downregulation. *Mol. Cell* 81, 1013–1026
41. Lis, J.T. *et al.* (2000) P-TEFb kinase recruitment and function at heat shock loci. *Genes Dev.* 14, 792–803
42. Wu, C.H. *et al.* (2003) NELF and DSIF cause promoter proximal pausing on the hsp70 promoter in *Drosophila*. *Genes Dev.* 17, 1402–1414
43. Chen, F.X. *et al.* (2017) PAF1 regulation of promoter-proximal pause release via enhancer activation. *Science* 357, 1294–1298
44. Vos, S.M. *et al.* (2018) Structure of activated transcription complex Pol II-DSIF-PAF-SPT6. *Nature* 560, 607–612
45. Mahat, D.B. and Lis, J.T. (2017) Use of conditioned media is critical for studies of regulation in response to rapid heat shock. *Cell Stress Chaperones* 22, 155–162
46. Alberti, S. and Hyman, A.A. (2021) Biomolecular condensates at the nexus of cellular stress, protein aggregation disease and ageing. *Nat. Rev. Mol. Cell Biol.* 22, 196–213
47. Biamonti, G. and Vourc'h, C. (2010) Nuclear stress bodies. *Cold Spring Harb. Perspect. Biol.* 2, a000695
48. Onoguchi-Mizutani, R. *et al.* (2021) Identification of a heat-inducible novel nuclear body containing the long noncoding RNA MALAT1. *J. Cell Sci.* 134, jcs.253559
49. Ray, J. *et al.* (2019) Chromatin conformation remains stable upon extensive transcriptional changes driven by heat shock. *Proc. Natl. Acad. Sci. U. S. A.* 116, 19431–19439
50. Li, L. *et al.* (2015) Widespread rearrangement of 3D chromatin organization underlies polycomb-mediated stress-induced silencing. *Mol. Cell* 58, 216–231
51. Lyu, X. *et al.* (2018) Architectural proteins and pluripotency factors cooperate to orchestrate the transcriptional response of hESCs to temperature stress. *Mol. Cell* 71, 940–955
52. Protter, D.S.W. and Parker, R. (2016) Principles and properties of stress granules. *Trends Cell Biol.* 26, 668–679
53. Cirillo, L. *et al.* (2020) UBAP2L forms distinct cores that act in nucleating stress granules upstream of G3BP1. *Curr. Biol.* 30, 698–707.e696
54. Lim, B. and Levine, M.S. (2021) Enhancer-promoter communication: hubs or loops? *Curr. Opin. Genet. Dev.* 67, 5–9

55. Niskanen, E.A. *et al.* (2015) Global SUMOylation on active chromatin is an acute heat stress response restricting transcription. *Genome Biol.* 16, 153
56. Niskanen, E.A. and Palvimo, J.J. (2017) Chromatin SUMOylation in heat stress: to protect, pause and organise?: SUMO stress response on chromatin. *Bioessays* 39, 1600263
57. Seifert, A. *et al.* (2015) Proteotoxic stress reprograms the chromatin landscape of SUMO modification. *Sci. Signal.* 8, rs7
58. Teves, S.S. and Henikoff, S. (2013) The heat shock response: a case study of chromatin dynamics in gene regulation. *Biochem. Cell Biol.* 91, 42–48
59. Cernilogar, F.M. *et al.* (2011) Chromatin-associated RNA interference components contribute to transcriptional regulation in *Drosophila*. *Nature* 480, 391–395
60. Rao, J.N. *et al.* (2006) Structural studies on the RNA-recognition motif of NELF E, a cellular negative transcription elongation factor involved in the regulation of HIV transcription. *Biochem. J.* 400, 449–456
61. Walters, R.D. *et al.* (2009) InvAluable junk: the cellular impact and function of Alu and B2 RNAs. *IUBMB Life* 61, 831–837
62. Yakovchuk, P. *et al.* (2009) B2 RNA and Alu RNA repress transcription by disrupting contacts between RNA polymerase II and promoter DNA within assembled complexes. *Proc. Natl. Acad. Sci. U. S. A.* 106, 5569–5574
63. Liu, W.M. *et al.* (1995) Cell stress and translational inhibitors transiently increase the abundance of mammalian SINE transcripts. *Nucleic Acids Res.* 23, 1758–1765
64. Pizzinga, M. *et al.* (2020) The cell stress response: extreme times call for post-transcriptional measures. *Wiley Interdiscip Rev RNA* 11, e1578
65. Brancolini, C. and Iuliano, L. (2020) Proteotoxic stress and cell death in cancer cells. *Cancers* 12, 2385
66. Klaips, C.L. *et al.* (2018) Pathways of cellular proteostasis in aging and disease. *J. Cell Biol.* 217, 51–63
67. Hetz, C. (2021) Adapting the proteostasis capacity to sustain brain healthspan. *Cell* 184, 1545–1560
68. Mallucci, G.R. *et al.* (2020) Developing therapies for neurodegenerative disorders: insights from protein aggregation and cellular stress responses. *Annu. Rev. Cell Dev. Biol.* 36, 165–189
69. Pavitt, G.D. (2018) Regulation of translation initiation factor eIF2B at the hub of the integrated stress response. *Wiley Interdiscip. Rev. RNA* 9, e1491
70. Shacham, T. *et al.* (2021) PERK pathway and neurodegenerative disease: to inhibit or to activate? *Biomolecules* 11, 354
71. Gaglia, G. *et al.* (2020) HSF1 phase transition mediates stress adaptation and cell fate decisions. *Nat. Cell Biol.* 22, 151–158
72. Mähl, P. *et al.* (1989) Rapid effect of heat shock on two heterogeneous nuclear ribonucleoprotein-associated antigens in HeLa cells. *J. Cell Biol.* 109, 1921–1935
73. Erhard, F. *et al.* (2019) scSLAM-seq reveals core features of transcription dynamics in single cells. *Nature* 571, 419–423
74. Vihervaara, A. *et al.* (2021) Stress-induced transcriptional memory accelerates promoter-proximal pause release and decelerates termination over mitotic divisions. *Mol. Cell* 81, 1715–1731.e6
75. Ninomiya, K. *et al.* (2021) m6^{A} modification of HSATIII lncRNAs regulates temperature-dependent splicing. *EMBO J.* 40, e107976
76. Sawarkar, R. *et al.* (2012) Hsp90 globally targets paused RNA polymerase to regulate gene expression in response to environmental stimuli. *Cell* 149, 807–818