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Review

Transcriptional reprogramming at the intersection of the heat shock response and proteostasis

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SUMMARY

Cellular homeostasis is constantly challenged by a myriad of extrinsic and intrinsic stressors. To mitigate the stress-induced damage, cells activate transient survival programs. The heat shock response (HSR) is an evolutionarily well-conserved survival program that is activated in response to proteotoxic stress. The HSR encompasses a dual regulation of transcription, characterized by rapid activation of genes encoding molecular chaperones and concomitant global attenuation of non-chaperone genes. Recent genome-wide approaches have delineated the molecular depth of stress-induced transcriptional reprogramming. The dramatic rewiring of gene and enhancer networks is driven by key transcription factors, including heat shock factors (HSFs), that together with chromatin-modifying enzymes remodel the 3D chromatin architecture, determining the selection of either gene activation or repression. Here, we highlight the current advancements of molecular mechanisms driving transcriptional reprogramming during acute heat stress. We also discuss the emerging implications of HSF-mediated stress signaling in the context of physiological and pathological conditions.

INTRODUCTION

Cells and organisms are constantly exposed to extrinsic and intrinsic stressors that endanger homeostasis. Such stress can be induced by hypoxia, toxins, mechanical stimuli, or elevated temperatures, which all compromise cell structure and function by causing macromolecular damage. To survive protein-damaging stress exposures, cells launch survival programs that mitigate stress-induced perturbations in protein homeostasis, i.e., proteostasis. These survival programs are characterized by the activation of rapid and transient transcriptional reprogramming that aims at adjusting RNA and protein synthesis, metabolic state, and the structural integrity of the cell[1] (Figure 1A). The stress-induced gene expression programs display a dual regulation, which is highlighted by a global stress-induced transcriptional attenuation (SITA),[2] while transcription of a subset of stress-protective genes is strongly induced (Figure 1B). The stress-induced transcriptional rewiring is orchestrated by a distinct selection of chromatin-binding regulators that respond to various acute and chronic stress cues. Exceeding the cellular survival capacity with prolonged or chronic stress can result in irreversible disruption of proteostasis and thereby threaten the survival of the whole organism. The cellular stress burden increases during aging and pathological conditions, such as cancer and neurodegeneration, highlighting the importance of properly regulated stress responses in organismal health.

The heat shock response (HSR) is a well-conserved and widely explored cell survival program that is activated in response to cytosolic protein damage. Upon acute heat stress inducing the HSR, cells activate the transcription of genes encoding molecular chaperones, such as heat shock proteins (HSPs), which maintain proteostasis by assisting protein folding, preventing protein aggregation, and directing damaged proteins for degradation.[3] Besides the upregulation of chaperones, cells decelerate proliferation and undergo morphological changes in response to thermal stress. This is ensured, for example, by stress-induced activation of cytoskeletal genes[4] and phosphorylation-dependent maintenance of the nuclear lamina.[5] An integral part of the HSR is also the genome-wide repression of thousands of genes, which could favor a proper coordination and distribution of cellular resources upon acute damage.

The rapid transcriptional upregulation of chaperone genes is mediated by heat shock transcription factors (HSFs) (Figure 1B). HSFs are highly conserved across species and analogous HSF-dependent transcriptional survival programs have been identified in all metazoans.[6,7] The activation-attenuation cycle of HSF1 is tightly controlled by HSPs, which maintain HSF1 inactive in the absence of stress. According to the chaperone titration model, stress-induced accumulation of misfolded proteins titrates the HSPs away from HSF1, allowing HSF1 trimerization, accumulation in the nucleus, and binding to its consensus target sequences called the heat shock elements (HSEs) (Figure 1B). The high variability in HSE architecture and sequence composition allows HSF binding to a diverse set of target genes.[4,8–11] The domain structures and post-translational modifications (PTMs) regulating the activity of HSFs are described in detail in recent reviews.[6,7,12,13] At their target loci, HSFs promote the recruitment of the transcription machinery and chromatin remodelers and enable the release

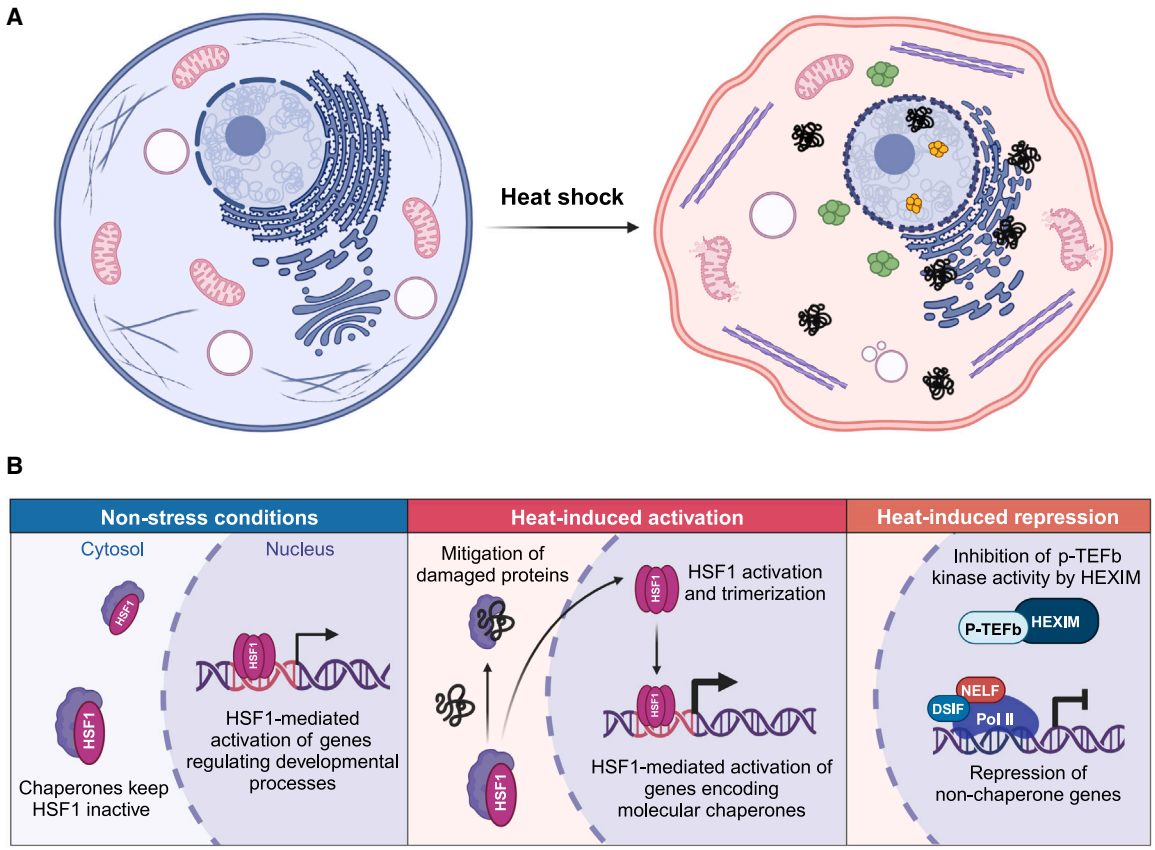

Figure 1. Cellular and transcriptional changes during acute heat stress

(A) Cellular adaptation in response to heat exposure. Heat shock leads to aggregation of misfolded proteins (black) in the cytosol and the nucleus. The endoplasmic reticulum and the Golgi complex (dark blue) are fragmented and disassembled. Mitochondria (pink) and lysosomes (white) decrease in number and their integrity is compromised. Membraneless assemblies are formed in the cytosol (stress granules, green) and in the nucleus (transcriptional condensates, yellow). Heat stress also induces structural damage, as demonstrated by reorganization of actin filaments into stress fibers (purple) and rearrangement of other cytoskeletal components (data not shown). The plasma membrane morphology is altered and there is an increase in membrane permeability.

(B) Transcriptional changes in response to heat stress. Under non-stress conditions, molecular chaperones, such as HSPs, keep the majority of HSF1 in an inactive monomeric state in the cytosol. The active pool of HSF1 regulates the expression of genes affecting developmental processes, including gametogenesis, aging, and lifespan. Exposure to heat increases the number of misfolded and aggregated proteins, leading to a rapid release of HSF1 from chaperones. Subsequently, HSF1 trimerizes, concentrates in the nucleus, and binds to its consensus target sequences, activating the expression of heat-inducible genes encoding molecular chaperones. Concomitantly, non-chaperone genes undergo global transcriptional repression. This stress-induced transcriptional attenuation (SITA) occurs due to decreased Pol II pause-release, which is caused by inactivation of the positive elongation factor, P-TEFb, by its negative regulator, HEXIM. Inhibition of P-TEFb kinase activity leads to loss of negative elongation factor (NELF) and DSIF phosphorylation, preventing Pol II release.

of promoter-proximal RNA polymerase II (Pol II) into productive elongation.[14–16] Removal of stress and reacquisition of proteostasis liberate HSPs, which participate in the repression of the transcriptional activity of HSF1 and lower the affinity of HSF1 for DNA.[17,18] Due to the robustness, fast dynamics, and high conservation of the regulatory components, the HSF-dependent HSR has provided an instrumental model to study the stress-inducible reprogramming of transcription. Together with the recent advances in genome-wide methods, the use of acute heat shock in cell-based model systems has enabled the analysis and identification of temporal changes occurring in gene and enhancer networks during cell stress. Despite these significant and groundbreaking advancements, our understanding of stress signaling in whole tissues or organisms, composed of multiple cell types, remains incomplete. Intriguingly, the physiological roles of many stress-inducible transcription factors, including HSFs, beyond acute stress responses is beginning to emerge. In this review, we highlight the recent advancements of the molecular mechanisms that drive stress-induced transcriptional reprogramming and discuss its implications in human physiology and pathology.

TRANSCRIPTIONAL ACTIVATION OF STRESS-RESPONSIVE GENES

The triumphant application of genome-wide techniques has expanded our knowledge of the extensive rewiring of transcription during stress. Methods such as global run-on sequencing (GRO-seq), precision run-on sequencing (PRO-seq), regulatory-element detection from GRO-seq (dREG), and precision run-on coupled to immuno-precipitation sequencing (PRO-IP-seq) map the exact sites of transcribing Pol II and have thereby enabled quantitative high-resolution analysis of transcription.[19–22] Multiple comprehensive reviews on the techniques have been recently published.[23–25] According to the current view, the mechanisms driving

stress-induced reprogramming engage a cascade of regulatory steps, including chromatin opening, assembly of the pre-initiation complex (PIC), initiation of transcription, promoter-proximal pausing of Pol II, and transcriptional elongation and termination (Figure 2A). Here, we will discuss these molecular steps in the context of acute heat stress.

Physical access to DNA is a universal requirement of transcription. The accessible chromatin landscape is continuously changing in response to intracellular and extracellular cues and thus reflects the stimuli that the cell is exposed to. The highly dynamic regulation of the chromatin landscape is coordinated by epigenetic changes mediated by chromatin-binding factors and histone modifying enzymes, which together organize the open chromatin regions across the genome.[26] Intriguingly, the majority of heat-inducible genes are in an open chromatin state already prior to stress exposure.[11,27–29] In *Drosophila*, the nucleosome-free state is maintained by the GAGA-associated factor (GAF), which binds GAGA-rich sequences in the promoters of heat-inducible genes. Upon binding, GAF interacts with chromatin remodelers and promotes sliding of nucleosomes through interaction with the nucleosome remodeling factor (NURF).[29] In mammalian cells, the nucleosome-free regions at heat-inducible promoters are maintained by HSF1 interacting with several proteins, such as FAcilitates Chromatin Transcription (FACT), replication protein A (RPA),[30] the SWI/SNF complex,[15] and Tip60.[31] Stress stimuli increase acetylation of histones H3 and H4, which loosens the interaction between histones and DNA, and is typically indicative of active transcription at promoters of genes and enhancer elements.[11,32]

PAUSE-RELEASE OF POL II, TRANSCRIPTIONAL ELONGATION, AND TERMINATION

Promoter-proximal pausing of Pol II is a critical quality control step in the early phases of transcription (Figure 2A). General transcription factors (GTFs) recruit Pol II at the transcription start site (TSS) of promoters to form the PIC, which initiates transcription. However, after transcribing 25–50 nucleotides downstream of the TSS, Pol II is temporarily locked into a promoter-proximal paused state before initiating transcription of a gene. As of today, a vast variety of factors regulating Pol II pause and release have been identified.[16] The paused Pol II is protected against premature elongation by negative elongation factor (NELF) and heterodimeric DRB-sensitivity-inducing factor (DSIF), which stabilize the Pol II complex and increase its residence time at the pause site. The release of paused Pol II is triggered by positive transcription elongation factors, particularly P-TEFb, a kinase that phosphorylates multiple proteins associated with the transcription machinery. Activation of heat-inducible genes causes dissociation of NELF from the genes,[33] but the depletion of NELF does not alter the kinetics of heat shock gene activation.[34] Phosphorylation of DSIF is considered as the main mechanism enabling Pol II release, [35] whereas the role of NELF is to promote the attenuation of the HSR by re-establishing Pol II pausing.[34]

Releasing Pol II from its paused state initiates transcriptional elongation and is triggered by the kinase activity of the P-TEFb complex. Upon heat shock, activated HSF1 binds to the HSEs of heat-inducible genes, together with chromatin remodelers and P-TEFb, leading to an increased rate of Pol II release into productive elongation (Figure 2A). Mechanistically, P-TEFb is recruited to the promoter either by HSF1[14] or RNA-binding motif protein 7.[36] P-TEFb phosphorylates NELF, DSIF, and the C-terminal domain of Pol II, which is required for dissociation of NELF from the chromatin. The phosphorylation of DSIF transforms it into a positive elongation factor, switching its function from supporting Pol II residency at the pause site to promoting transcriptional elongation.[37] The positioning and size of the Pol II complex causes a steric hindrance, thereby inhibiting the entry of new Pol II molecules to the TSS and making Pol II release into productive elongation a rate-limiting step of gene transcription.[38]

Gene size is important for transcriptional timing and provides a mechanism for temporal regulation of gene expression. The elongation rate of Pol II is affected by gene length. A high elongation rate correlates with longer genes, while a low elongation rate is observed in exon-dense genes that generally display more complex sequences.[39] Pol II has been shown to transcribe faster across introns to enable synthesis of long genes. The accelerated elongation rate depends on the association of a specific elongation complex (U1), which regulates the transcription of full-length transcripts.[40] Termination of transcription occurs when Pol II approaches the end of the gene, where the elongation rate slows down, and the transcript is cleaved at the poly(A) signal and polyadenylated to release the newly synthesized mRNA (Figure 2A). Termination can occur at several different sites along the transcribed gene, either with the purpose of generating differential transcripts with specific regulatory properties or of preventing aberrant transcript formation.[41] Reduced termination could function as a mechanism to restrict production of new proteins when energy reserves are depleted due to constantly occurring stress. In contrast, premature termination can serve as an important mechanism for transcriptional control, as the prevention of full-length transcripts is a powerful tool to negatively regulate gene expression. Acute heat stress has been shown to increase the elongation rate of Pol II but, importantly, the processivity was decreased due to premature transcript termination at cryptic, intronic polyadenylation sites, plausibly through disrupted U1 telescripting.[42] This led to a massive production and accumulation of short, unannotated nascent transcripts in the nucleus, suggesting that premature termination of transcription contributes to the downregulation of genes upon heat shock. Premature termination can also be mediated by the Integrator complex, which comprises both endonuclease and phosphatase activities. Depletion of Integrator endonuclease (INTS11) increased the amount of active early elongation complexes, which failed to achieve optimal elongation rates, resulting in only short transcripts.[43] Therefore, INTS11 likely has a function across all Pol II-transcribed loci with differential effects on genes that are reflective of transcript length rather than the specificity of Integrator.

STRESS-INDUCED READTHROUGH TRANSCRIPTION

Various types of stress, such as high temperatures or viral infections, can induce Pol II transcription past the normal termination site into neighboring genes, a process termed readthrough transcription.[44,45] Readthrough transcription generates long non-coding RNAs downstream of gene-containing transcripts (DoGs), which can extend up to thousands of kilobases beyond the gene

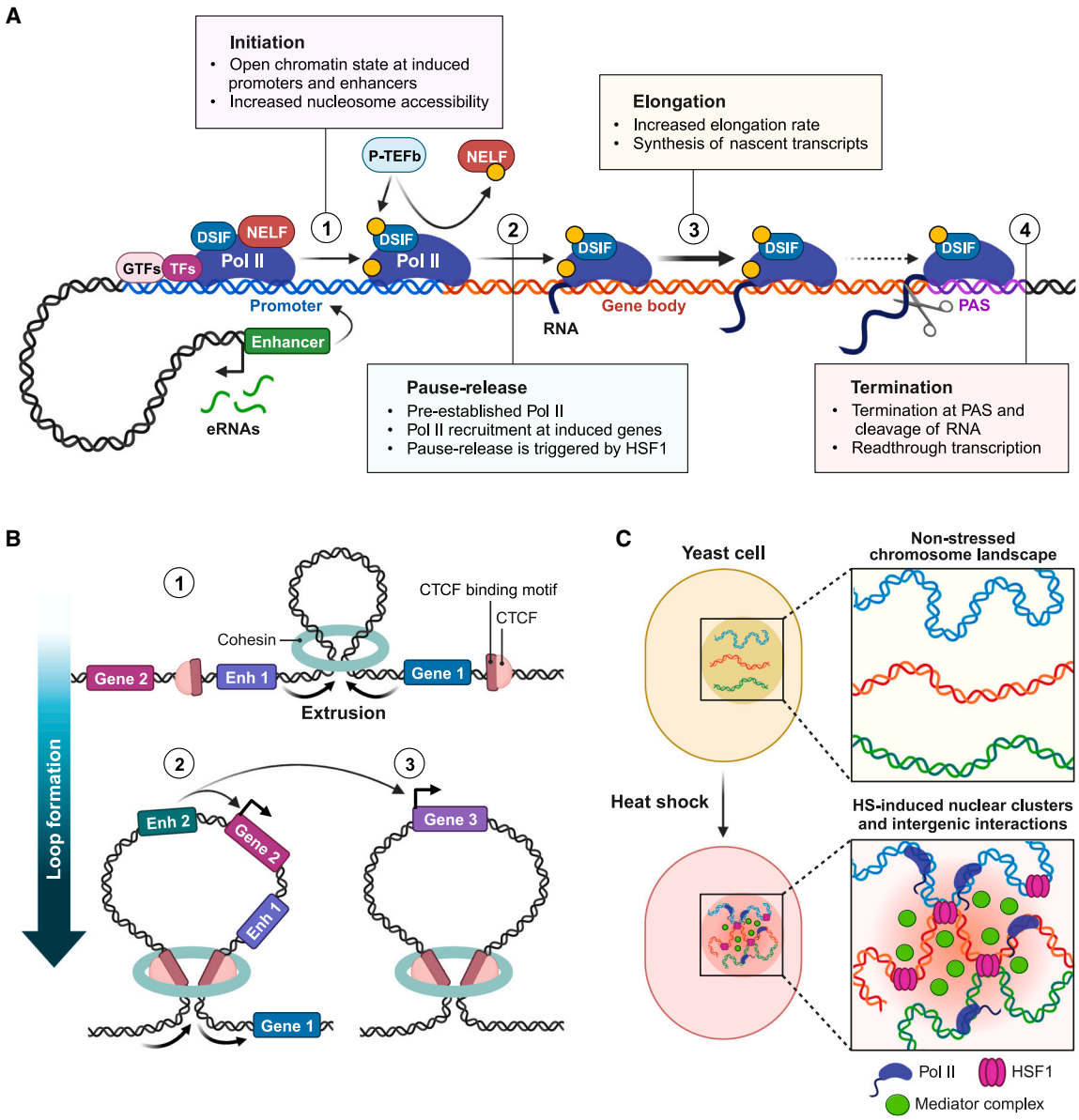

Figure 2. Heat-induced changes in transcriptional steps and chromatin architecture

(A) Exposure to heat stress induces changes in all steps of transcription (1–4). Upon activation of heat-inducible genes, general transcription factors (GEFs) and transcription factors (TFs) bind to the promoter and recruit the positive elongation factor P-TEFb, which phosphorylates (yellow) Pol II, DSIF, and NELF. Phosphorylation of DSIF turns it into a positive elongation factor, while phosphorylated NELF dissociates from the transcription complex, releasing Pol II into productive elongation. Enhancers (green) are distal regulatory elements that can stimulate gene activity and are connected to the target promoters through chromatin looping that is carried out by the Mediator complex (data not shown). Enhancers can be transcribed into short non-coding enhancer RNAs (eRNAs). Heat shock can increase the elongation rate of transcribing Pol II (thick arrow). The elongation rate of Pol II slows down (dotted arrow) at the end of the gene, and the nascent transcript is cleaved at the poly(A) signal (PAS), denoting termination of transcription. In some instances, readthrough transcription can occur into neighboring genes (not shown).

(B) Stress-induced formation of chromatin loops is defined by topologically associated domains (TADs). The loop extrusion model encompasses cohesin binding and pulling of chromatin to grow a loop (1). The interaction between cohesin and chromatin-bound CTCF at the TAD boundary stabilizes the structure, enabling contact between enhancers (Enh) and genes located within the same TAD (Enh 1 and Gene 1). Heat shock can alter the localization CTCF, which generates new stress-induced TADs (Enh 2 and Gene 2) (2). In *Drosophila* cells, exposure to heat also allows the formation of inter-TAD connections (Enh 2 and Gene 3) (3).

(C) Heat shock (HS) induces the formation of intergenic connections in yeast. The profound remodeling of the chromatin architecture that enables interactions over large distances is mediated by the formation of stress-inducible nuclear transcriptional condensates constituted of Pol II (blue), HSF1 (pink), and the Mediator complex (green).

end.[44,46] In contrast to other non-coding RNAs, DoG transcription is initiated at the promoter of a protein-coding gene. Readthrough transcription can occur due to the loss of transcription termination,[44,47] reduced frequency of strong polyadenylation signals in DoG-producing genes,[48] or the absence of nuclear polyadenylation-binding protein (NAB2) and the Integrator complex.[49,50] Production of DoGs can result in read-in transcription into downstream genes and modulate their expression through transcriptional interference. Recently, read-in genes were demonstrated to display significantly reduced translation and marked intron retention during stress, portraying a relationship between the two processes.[45] Widespread intron retention in response to heat shock has previously been reported to accumulate polyadenylated, stable, intron-containing mRNAs in the nucleus.[51] In heat-shocked cells, readthrough transcription of a subset of genes was shown to correlate with HSF1 binding to their promoters,[52] but additional studies are required to determine how the process is regulated. Heat stress leads to the loss of Pol II at the 3′ ends of protein-coding genes, but polymerase occupancy is increased over large distances downstream of the 3′ ends. A 1-h recovery time was sufficient to restore the typical pattern of Pol II occupancy, suggesting that heat stress induces a reversible loss of normal termination.[53] Reduced termination may be important for controlling the cellular response to heat shock as it leads to widespread genomic repression of gene transcription. Furthermore, readthrough transcription could function as a regulatory step to maintain open chromatin during cellular stress, allowing rapid reversion to normal Pol II termination when stress signaling seizes. However, several critical questions are yet to be answered regarding the production and functionality of readthrough products, including the specific molecular mechanisms regulating readthrough transcription and the fate of the generated readthrough products.

TRANSCRIPTIONAL REPRESSION OF STRESS-RESPONSIVE GENES

Pol II pause-release is the central mechanism allowing transcriptional activation, but it also serves as the key step of restriction if it remains in the paused state. This type of dual regulation is evident under acute heat stress, where a subset of stress-protective genes (10%) is induced, while the majority of active genes (55%) undergo a general transcriptional lockdown[4] (Figure 1B). The massive downregulation of transcription is termed SITA.[2,54] The functional role of the overall decrease in RNA and protein production is considered to spare the cell's proteostasis capacity. Many studies have demonstrated that genome-wide transcriptional repression is not caused by decreased Pol II levels at the promoters of downregulated genes, but instead diminished transcription of the gene body, reflective of increased Pol II pausing.[2,4,11] Indeed, both increased binding of NELF and inhibition of P-TEFb activity, through induced interaction with its negative regulator HEXIM, have been observed in cells exposed to heat shock[2,55] (Figure 1B). These results suggest that regulation of Pol II pause-release is the predominant checkpoint influencing transcriptional repression.

An outstanding question is how molecular chaperones escape SITA. Because increased NELF binding and P-TEFb are also essential for HSF-dependent activation of chaperone genes,[14,33] it is plausible that there are additional molecular mechanisms governing stress-inducible transcription. Heat shock increases NELF abundance but also Pol II recruitment at the promoters of chaperone genes, which would maintain an effective ratio, thereby allowing Pol II transcription despite high levels of NELF. In contrast, the level of Pol II at SITA target genes is not increased, which shifts the ratio to favor NELF-mediated repression.[55] Although acute heat stress leads to HEXIM-mediated inactivation of P-TEFb, there might be a remanent pool of active P-TEFb that is accessible to HSFs. Further studies are required to uncover how specifically stress-inducible transcription factors, but not other transcription factors, gain access to the active P-TEFb complex.

TRANSCRIPTIONAL REGULATION OF ENHANCERS DURING STRESS

Enhancers are distal *cis*-regulatory DNA elements that have a central role in orchestrating the spatiotemporal regulation of gene expression programs. Induction of target gene activity occurs through DNA-looping, executed by the Mediator complex that forms a connection between the enhancer-bound transcription factors and components of PIC at the promoter[56] (Figures 2A and 2B). The composition of the transcriptional machinery at enhancers is comparable to that of promoters, indicating that similar molecular mechanisms coordinate the activity of these regulatory elements. Active enhancers are subjected to several chromatin modifications, such as histone H3K27Ac.[57] Genome-wide analyses have been fundamental in delineating the kinetics and molecular determinants of these distal regulatory elements. Moreover, it has been revealed that enhancers are transcribed bidirectionally into short non-coding enhancer RNAs (eRNAs) (Figure 2A), which influence transcription and chromatin looping through yet unknown mechanisms.[56] Similarly to gene reprogramming, transcription of enhancers undergoes extensive changes upon acute stress. Heat shock increases the overall occupancy of Pol II at enhancers, which is coordinated at the level of Pol II pause-release.[11] Intriguingly, when human embryonic stem cells were exposed to elevated temperatures, the chromatin-binding pattern of HSF1 and pluripotency factors (Kruppel-like factor 4, NANOG, and octamer-binding factor) were dramatically altered, leading to rewired tethering of enhancers to their cognate promoters.[58] These results suggest that the stress-specific transcription factors, together with lineage-specific factors, coordinate the genome-wide alterations of the enhancer landscape.

Transcriptional reprogramming during heat shock is well established, but the molecular mechanisms regulating gene expression during other types of stress are less understood. HSF1 has been reported to increase the transcription of FOXO3 through an intronic enhancer in its gene body in response to heat shock and oxidative stress,[59] suggesting that there are shared targets upon distinct types of stress. A recent study provided more insights into transcriptional rewiring upon stress by systematically investigating how two members of the HSF family, i.e., HSF1 and HSF2, coordinate gene transcription in cells exposed to either heat shock or oxidative stress.[60] Although, these transcription factors triggered Pol II pause-release by binding to stress-inducible promoters and enhancers, they regulated the target genes in a stress-specific manner. For example, HSF1 was found to bind chaperone genes upon both stresses, but it was capable of

activating transcription only in heat-shocked cells. In contrast, under conditions causing redox imbalance, HSF1 occupied unique promoters and enhancers to activate genes specific for oxidative stress.[60] Thus, enhancer networks have emerged as a critical regulatory layer of transcription, allowing spatiotemporal control and fine-tuning of gene expression programs. Currently, how the stress-contingent specificity between heat stress and oxidative stress is reached is unknown, but it is plausible that several mechanisms contribute to target gene selectivity. HSF2 is dispensable for the genome-wide stress inducibility but co-occupies the same enhancers and promoters as HSF1, indicating that the two factors cooperate to drive transcription during different types of stress. Intriguingly, the stress-type-specific binding cannot be explained by the target sequence because both HSFs bind to canonical HSEs. Instead, target gene selection could be orchestrated through recruitment of stress-inducible co-factors and protein modifications as well as changes in the chromatin architecture that together allow the HSFs to access unique binding sites depending on the stress stimuli.

HEAT-STRESS AND TRANSCRIPTIONAL MEMORY

Previous exposure to environmental stress stimuli can enhance the responsiveness of specific genes, generating an epigenetic transcriptional memory. This provides cells with an adaptive system, permitting faster transcriptional rewiring in response to periodic stress exposures that challenge cell survival.[61] Besides acting as a key step for the orchestration of transcription, the promoter-proximal Pol II pause-release is also important for the transcriptional memory that is retained over cell divisions. Heat-induced transcriptional rewiring is restored to the basal cell-type-specific program within hours of recovery from stress.[62] In mouse embryonic fibroblasts, a single exposure to heat shock primed an instant activation of a subset of genes in the daughter cells. The rapid responsiveness was constituted by an increase in promoter-proximal Pol II pausing and pause-release, which is indicative of accelerated transcriptional onset. In contrast, repeated exposures to heat stress in human erythroleukemia K562 cells was shown to encode a transcriptional memory that leads to reduced termination of transcription and, consequently, impaired recycling of Pol II to promoters and enhancers in the daughters of stressed cells.[62] Thus, the transcriptional memory following heat shock can be regulated at two critical sites, i.e., by increased Pol II pausing at promoter-proximal regions and reduced termination of transcription. As decreased Pol II release from promoter-proximal sites at heat-repressed genes is a reversible mechanism, gene transcription can be rapidly re-activated by inducing the pause-release. Pol II can maintain accessible promoter structure even during transient repression, including heat-induced repression, and restore genome-wide transcription without extensive chromatin remodeling. Such regulation would provide the cell with a rapid and efficient mechanism to ensure transcriptional recovery, which is critical for proteostasis.

THERMAL-STRESS-INDUCED REORGANIZATION OF THE CHROMATIN ARCHITECTURE

The eukaryotic genome is hierarchically organized into distinct structural loops and higher order domains. Regulatory signals are typically relayed via chromatin loops that facilitate transcription by reducing the effective distance between enhancers and their cognate promoters. Chromatin is organized in a three-dimensional (3D) structure with multiple topologically associating domains (TADs), which are self-interacting genomic regions, forming loops between gene promoters and enhancers.[63] TADs are architecturally defined by well-conserved boundaries highly enriched with CCCTC-binding factor (CTCF) and cohesin. According to the chromatin loop extrusion model (Figure 2B), cohesin binds and pulls chromatin to grow a loop until it encounters a chromatin-bound CTCF at the TAD boundary. The interaction between cohesin and CTCF at the TAD boundaries stabilizes the chromatin loop structure.[64] The cohesin subunit RAD21 plays an important role in promoting chromatin loop extrusion.[65]

The effects of thermal stress on 3D chromatin conformation are still elusive. There is, however, evidence that in human H9 embryonic stem cells, stress leads to changes in the localization of CTCF and cohesin, resulting in the formation of new stress-mediated loops that regulate the interaction between enhancers and promoters[58] (Figure 2B). Similarly, in *Drosophila* Kc167 cells, CTCF and cohesin relocalize from the boundaries to enhancers and promoters during heat shock, thereby weakening the boundary architecture and inducing inter-TAD connections[66] (Figure 2B). The heat-induced disruption of TAD boundaries was found to allow the formation of Polycomb-complex-containing enhancer-promoter clusters that facilitate gene repression.[66] Another study demonstrated striking stability in chromatin compartments and TADs in both human and fly cells exposed to heat shock, reflecting an evolutionarily conserved mechanism that enables cells to respond rapidly to stress.[67] Accordingly, pre-established enhancer-promoter interactions have been reported under various conditions.[68,69] However, based on current literature, the role of TAD boundaries during heat stress remains inconclusive. It is possible that changes in TAD structures depend on the cell state and may occur only in specific subsets of genes and enhancers. For example, TADs disappear during mitosis,[70] TAD boundaries can be disrupted during oncogenesis,[71] and changes in loop structures as well as compartmental switching have been detected in differentiating and senescent cells.[72–74] These results suggest that both dynamic and stable enhancer-promoter contacts contribute to the spatiotemporal regulation of transcription. Further studies using physiologically relevant models representing different cell states are warranted to uncover the regulation of TADs during thermal stress.

HEAT-INDUCED TRANSCRIPTIONAL REWIRING THROUGH PTMs

The rapid transcriptional reprogramming upon heat shock is heavily supported PTMs of chromatin-associated proteins, such as histones, transcription factors, and epigenetic enzymes (Table 1). Apart from conjugation of different chemical groups, histones and other chromatin-associated proteins are frequently modified by ubiquitin and ubiquitin-like proteins, such as SUMOs and NEDDs.[75] Polyubiquitination is critical for the global transcriptional repression upon heat shock. Cells with functionally inhibited ubiquitin E1 activating enzyme show

Table 1. Examples of stress-inducible PTMs and their effects during acute heat stress

Examples of PTMs and substrates	Effect in response to heat shock	References
Acetylation of histones H3 and H4	stress-induced acetylation loosens chromatin structure to allow active transcription	Vihervaara et al.[11] and Mueller et al.[32]
Monoubiquitination of eEF1BδL	recruitment of P-TEFb at promoters of heat-induced genes	In et al.[76]
Neddylation	promotes protein aggregation and compromises ubiquitination to protect the nuclear ubiquitin-proteasome system from stress-induced dysfunction	Maghames et al.[83]
Neddylation + sumoylation	hybrid conjugates of NEDD8-SUMO2 chains accumulate in stress-induced nucleolar clusters	Lobato-Gil et al.[84]
Parylation	induces parylation, which leads to the loss of nucleosomes and induced transcription of HSP genes	Murawska et al.,[85] Fujimoto et al.,[86] and Huang and Kraus[87]
Phosphorylation + sumoylation of HSF1	fine-tunes the HSF1-mediated *trans*-activating capacity	Hietakangas et al.[78,79]
Polyubiquitination of nascent proteins	global transcriptional repression upon heat shock	Aprile-Garcia et al.[2]
Sumoylation of CTCF binding sites	heat shock decreases sumoylation	Niskanen et al.[77]
Sumoylation of chromatin	increases sumoylation at promoters and enhancers restricting transcription	Niskanen et al.[77]

impaired ability to downregulate transcription upon heat stress, connecting translation and ubiquitination with the stress-induced transcriptional response.[2] In contrast to poly-ubiquitination, which typically directs proteins for proteasomal degradation, monoubiquitination can alter the structure, activity, and binding of proteins. Monoubiquitination of the transcription factor eEF1BδL by the ubiquitin E3 ligase RNF20/40 recruits P-TEFb at the promoters of heat-responsive genes. Notably, eEF1BδL and RNF20/40 interact physically with HSF1 and synergistically promote the expression of heat-inducible genes.[76] The conjugation of SUMOs to chromatin-bound proteins is strongly induced in response to heat stress at active promoters and enhancers associated with several transcription factors, such as HSF1.[77] Upon heat shock, HSF1 undergoes rapid and transient phosphorylation-dependent sumoylation that fine-tunes its *trans*-activation capacity.[78,79] Recent *in vitro* data demonstrated that sumoylation occurs more efficiently on trimeric HSF1 than the monomeric form and without affecting the DNA-binding capacity. Thus, the sumoylation-mediated attenuation of HSF1 likely occurs through recruitment of co-repressors or impaired interaction between HSF1 and the transcription machinery.[80] Sumoylation provides a mechanism for restricting the transcriptional activity of heat-induced genes, albeit the molecular details and the exact sumoylation target proteins mediating the repression remain to be identified.[7] Intriguingly, heat shock was shown to decrease sumoylation of regions with binding sites for CTCF.[77] CTCF is an important regulator of the 3D structure of chromatin, suggesting that sumoylation might participate in the coordination of chromatin architecture. Although several components of the transcription machinery have been found to undergo sumoylation,[81,82] its regulatory impact on transcription initiation, elongation, and termination remains to be established.

Another ubiquitin-like modification, neddylation, promotes the function and stability of proteins. Neddylation increases in response to heat shock and promotes the formation of protein clusters in the nucleus, which is a transient defense mechanism against proteotoxic stress. Mechanistically, NEDD8 compromises ubiquitination, thereby protecting the nuclear ubiquitin-proteasome system from stress-induced dysfunction.[83] Interestingly, NEDDs can form hybrid conjugates together with ubiquitin or SUMOs, and NEDD8-SUMO2 chains were recently shown to accumulate into nucleolar clusters—called nucleolus-related inclusions—upon heat shock.[84] Collectively, these studies demonstrate that ubiquitin and the ubiquitin-like modifier proteins can crosstalk and regulate the nuclear proteostasis network during acute stress. Future studies will focus on elucidating the impact of neddylation-mediated condensation and hybrid conjugates on stress-induced transcriptional reprogramming.

Chromatin-associated proteins can also be modified by other chemical moieties, such as long branched ADP-ribose polymers. In heat-shocked cells, poly(ADP-ribosyl)ation, also called parylation, has been shown to induce the expression of HSP genes by generating an insulated region for transcription.[31,85–87] Exposure to heat stress leads to redistribution of poly(ADP-ribose) polymerase 1 (PARP1) throughout the HSP70 locus, where increased parylation facilitates HSF1 binding and the recruitment of co-activators and chromatin remodeling proteins.[86] In contrast, PARP1 contributes to stress-mediated downregulation of gene expression by parylating the mRNA 3′ processing enzyme, poly(A) polymerase, thereby inhibiting its enzymatic activity required for stabilizing the newly synthesized pre-mRNAs.[88]

Currently, it is not known how PARP1 distinguishes between genes that are either positively or negatively regulated by stress, but the mechanism likely involves the tight coordination of PARP1 localization at specific genomic regions.

STRESS-INDUCED CONDENSATES IN TRANSCRIPTIONAL REGULATION

Biomolecular condensates are membraneless assemblies of concentrated biopolymers, typically formed through liquid-liquid phase separation in the cytosol or in the nucleus[89] (Figure 1A). Heat stress can lead to large-scale alterations in the 3D organization of the genome. In yeast, heat shock induces profound remodeling of the chromatin architecture, allowing heat-inducible genes to interact with each other over large distances and even between different chromosomes.[90] Recent results have demonstrated that the formation of stress-inducible transcriptional condensates, comprising HSF1, the Mediator complex, and Pol II, provides the underlying mechanism for chromatin remodeling and intergenic interactions[91] (Figure 2C). Such transcriptional clusters are typically established and maintained by multivalent interactions mediated by intrinsically disordered protein domains.[92] The molecular determinants endowing stress inducibility and capacity for intergenic interactions are HSP70 and the intrinsically disordered domain of HSF1, which repress or promote clustering, respectively.[91] These results indicate that dynamic spatiotemporal condensates are critical for the control of eukaryotic gene expression.

In human cells, nuclear condensates have been proposed to be involved in transcriptional regulation, as Pol II recruitment to promoters coincides with so-called promoter condensates enriched in transcription factors and transcriptional co-activators. Formation of nuclear condensates likely occurs through liquid-liquid phase separation of proteins that, similarly to the yeast model, contain intrinsically disordered regions.[93] The ability of proteins to induce condensation through these intrinsically disordered domains is profoundly affected by PTMs. Particularly, the hyperphosphorylation of Pol II has been connected with the formation of nuclear condensates that regulate transcription initiation and RNA processing.[94,95] Heat shock induces the formation of small nuclear condensates where HSF1 is enriched at the loci of HSP genes, promoting their transcription. The phase-separation capacity of HSF1 is fine-tuned by the phosphorylation of specific sites located in the regulatory domain. In support of the findings in yeast, HSP70 disperses HSF1 condensates, which in turn attenuates target gene transcription and prevents further phase transition of HSF1,[96] evidencing that the mechanism is conserved from yeast to human. Intriguingly, acute heat stress was shown to induce nuclear condensation of NELF, which was causally linked to reduced Pol II elongation and proposed as a mechanism for the genome-wide transcriptional lockdown during SITA.[55] The condensation of NELF similarly requires multivalent intermolecular interactions between the disordered regions in subunit NELFA and NELFE.[97] Condensate formation requires stress-induced dephosphorylation and sumoylation of NELF,[55] suggesting that the stress inducibility of NELF condensates is regulated through PTMs. Further investigations are required to shed light on the involved signaling pathways that regulate the formation of nuclear condensates during stress exposure.

HSF-DRIVEN PROTEOSTASIS NETWORKS IN PHYSIOLOGY AND PATHOLOGY

Exposure to heat stress endangers cell survival by disrupting cellular protein homeostasis. Proteostasis is not, however, only challenged by extrinsic stressors but also by various physiological conditions, such as cell differentiation and development (Figure 3A). Similar to acute stress, differentiation is driven by global transcriptional reprogramming and chromatin modifications that alter the proteostasis network. For example, human embryonic stem cells undergo extensive chromatin reorganization during lineage specification,[72] whereas live imaging of mouse epidermis revealed that a gradual transition in chromatin compaction occurs in differentiating cells exiting the epidermal stem cell compartment.[98] During differentiation, cells undergo a dramatic shift in their gene and protein expression programs, which requires an extremely tight coordination of the proteostasis network. However, the mechanisms regulating the transcriptional reprogramming at the intersection of physiological and pathological cell fate decisions require further investigations.

HSF1 and HSF2 are the most extensively studied members of the mammalian HSF family. Early findings have already implicated both HSF1 and HSF2 as providing a key role in mammalian development, but the molecular details of this regulation have remained largely unknown. The protein levels of HSFs fluctuate during mouse embryogenesis, whereas adult animals deficient of HSFs display impaired gametogenesis and both male and female mice are sterile.[99] Intriguingly, in ants, long lifespan is maintained by increased HSP gene expression in gamergates, which is partly driven by a gamergate-specific upregulation of a truncated form of an HSF resembling the mammalian HSF2.[100] During normal brain development, HSF1 and HSF2 contribute to neuroepithelial integrity, synapse formation, and responses to proteotoxic stress,[101] whereas the lack of HSF2 causes ventricular deformation.[102,103] An example of abnormal convergence of stress and developmental signaling is provided by mouse corticogenesis, during which HSF2 forms homotrimers to promote normal neural migration. However, prenatal exposure to alcohol skews transcriptional programs and disrupts the migratory capacity of neural cells through forced HSF1-HSF2 heterotrimerization,[104] suggesting that stress signaling can overrun the developmental functions of HSFs. In response to acute neuronal stimuli, the gene encoding brain-derived neurotrophic factor was shown to be directly regulated by HSF1, indicating that HSF1 has an important role in neuronal protection and plasticity in the mouse hippocampus.[105] In the mouse brain, the neuroepithelial integrity during cerebral development depends on the acetyltransferase CBP/EP300-mediated stabilization of HSF2, which allows activation of HSF2 target genes, including N-cadherin and the subsequent promotion of neuronal stress resistance.[106] Intriguingly, HSF2 has been shown to enhance cell survival against proteotoxicity through the maintenance of cadherin-dependent cell-cell adhesions,[106,107] suggesting that the HSF-regulated survival

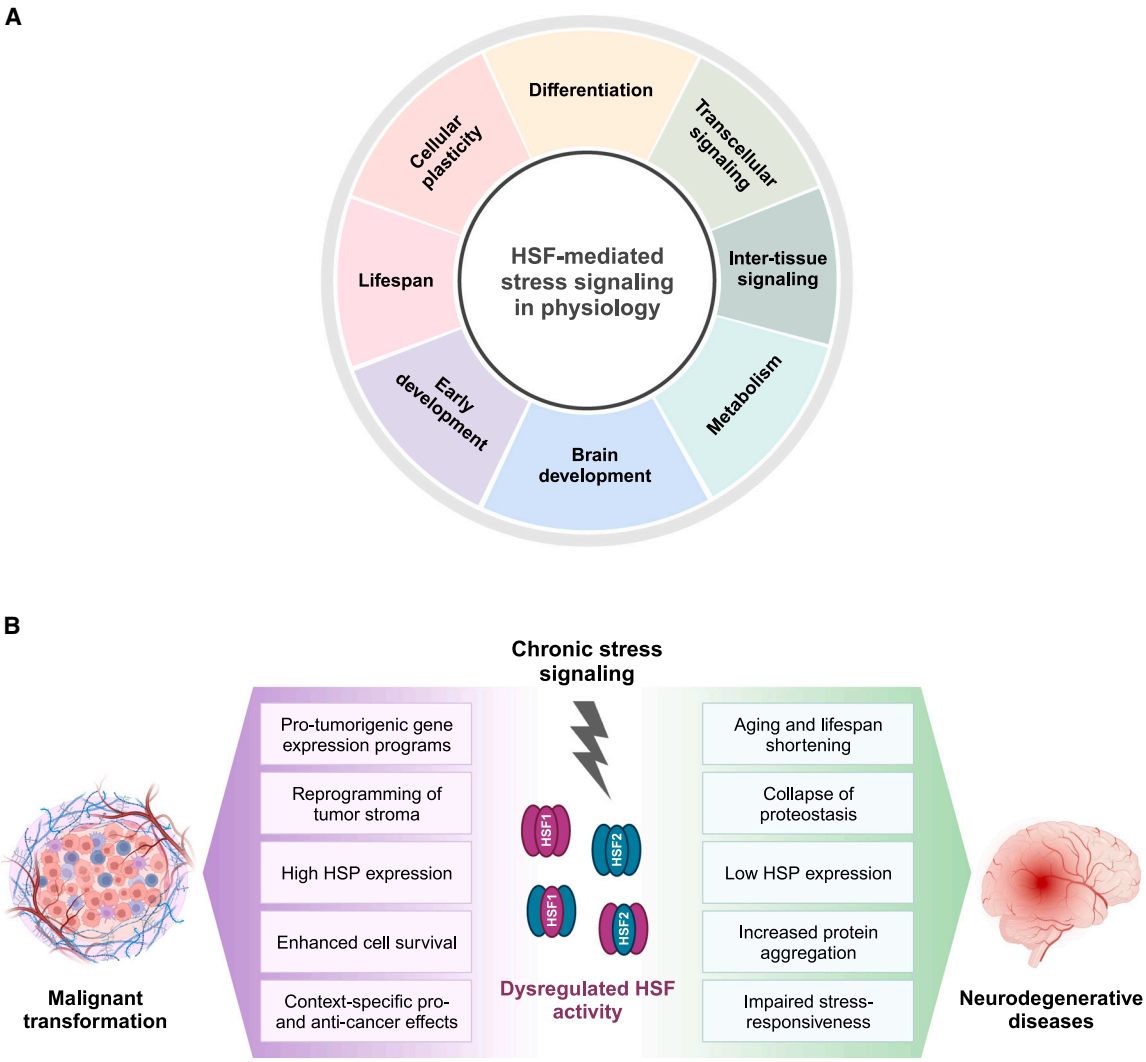

Figure 3. Heat shock factor-mediated stress signaling in physiology and pathology

(A) HSFs mediate stress signaling in a broad selection of physiological processes.

(B) Chronic stress signaling leads to dysregulation of HSF-mediated signaling. The effects of dysregulated HSFs have been primarily investigated in cancer and neurodegenerative diseases. High levels of HSF1 correlate with malignant transformation, while the function of HSF2 in tumorigenesis is context dependent. Cooperation of HSF1 and HSF2 can also promote disease progression in specific cancer types. In contrast, HSF-dependent collapse of proteostasis is evident in many neurodegenerative diseases. The function and stoichiometry of HSF1-HSF2 heterotrimers in pathology remain poorly understood.

mechanisms at the tissue level may be more diverse than originally anticipated.

The rapid activation of HSP gene transcription is a hallmark of HSF1-dependent HSR. In the context of organismal development, however, HSF1 drives a specific transcriptional program,[9] demonstrating that the HSF-driven transcription is tailored according to the cell and tissue type and the developmental stage. To date, only a few molecular mechanisms are known to underly this tailored program. For example, in *C. elegans*, the development-associated target gene selection is specified by a distinct promoter structure harboring a GC-rich motif in the proximity of the HSF1-binding site.[9] The GC-rich motifs are bound by E2F, which is a critical transcriptional coregulator responding to the cell cycle phase and the metabolic state of the cell. The functional interaction of E2F and HSF1 enables the coordination of energy production and protein quality control, indicating that the program selection must be able to respond to a variety of cellular signals. Also, the HSE architecture shows considerable variation in sequence, spacing, and orientation in mammalian cells,[8] adding yet another layer to the complexity of target gene selection. In heat-shocked cells, HSF1-dependent recruitment of the transcription machinery forces the expression of HSPs in otherwise repressed chromatin environment, but how HSF1 engages the transcriptional machinery in other HSF1-specific gene programs is still unknown.

Several recent findings indicate that cell-specific stress signals are communicated between tissues to maintain organismal homeostasis.[108] In *C. elegans*, the nervous system, comprising neurons and the supporting glial cells, is the main regulator of organismal proteostasis, and it has been shown to orchestrate

the systemic stress response.[109,110] For example, maternal neurons release serotonin to protect the vulnerable germ cells from damage and to ensure the survival of their progeny. Serotonin signaling acts through HSF1, which, upon stimulation, recruits the histone chaperone FACT to target promoters and induces the transcription of protective genes.[111] HSF1 has been shown to promote longevity by coordinating bone morphogenetic protein signaling along the gut-brain axis, suggesting that inter-tissue stress signaling is critical for balancing proteostasis during the lifespan of *C. elegans*.[112] The role of inter-tissue communication in organismal homeostasis is also evidenced by transcellular chaperone signaling, which can utilize an HSF1-independent mechanism to activate chaperone gene expression in distal tissues.[113] Moreover, transcellular signaling is evident in embryo-to-mother communication, linking the integrity of the developing progeny to the regulation of proteostasis in the parents.[114]

Organismal aging is hallmarked by the collapse of proteostasis, which is reflected in an increased predisposition to human pathologies such as cancer and neurodegenerative diseases[115] (Figure 3B). Cancer cells face an increased burden of misfolded proteins and harness the forces of stress signaling to enhance cell survival, whereas accumulation of protein aggregates and declined HSR is characteristic to neurodegeneration. Accordingly, HSF1 expression is significantly upregulated in many cancer types and correlates with metastasis and poor prognosis.[116] HSF1 drives cancer-specific transcriptional programs, both in the tumor cells and in the adjacent cancer-associated fibroblasts, to promote malignant progression.[117–120] In contrast, the expression of HSF2 varies in different malignancies and its function in cancer is still elusive. Nevertheless, high HSF2 levels have been linked with increased cell proliferation,[116,120] suggesting that the amount of HSF2 would reflect the growth potential of cancer. The age-dependent decay in HSF1 activity is tightly linked to neurodegenerative diseases that are manifested by transcriptional proteostasis decline, influencing chromatin architecture and dynamic gene expression programs[121,122] (Figure 3B). Using mouse models and human patient samples of Huntington's disease, which is characterized by aggregated polyglutamine repeats of huntingtin, it has been demonstrated that HSF1 is inappropriately targeted for ubiquitin-proteasomal degradation, leading to defective target gene expression.[123] Interestingly, knockout of HSF2 has been associated with the aggregation of polyglutamine proteins and lifespan shortening in a Huntington's disease mouse model,[124] indicating that HSF2 is required for maintaining the proteostasis machinery against polyglutamine aggregation. Whether specific HSF-dependent genes, beyond the HSR, that control lifespan are dysregulated during aging remains to be explored. Taken together, in view of current knowledge, a plethora of correlative findings demonstrate that HSFs are integrated in many common disease pathways, but the causality of underlying mechanisms remains to be established.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The cellular response to acute heat shock activates rapid and transient transcriptional programs to mitigate stress-induced damage. This transcriptional reprogramming harnesses dual regulation, which is defined by induction of stress-protective genes, especially those coding for molecular chaperones, and concomitant genome-wide repression of non-chaperone genes. The dramatic rewiring of gene and enhancer networks is driven by epigenetic modifications and stress-inducible transcription factors, including HSFs, that alter the 3D chromatin architecture. Although the same regulators are involved in both gene activation and repression, further studies are required to understand how a distinct subset of genes, such as those encoding chaperones, can escape the global transcriptional silencing. Similar to acute stress, proteostasis is also challenged under physiological processes, including differentiation and development, where HSFs have emerged as crucial determinants. HSFs occupy an extensive repertoire of target loci also in the absence of stress, but whether this is functionally relevant for transcriptional regulation remains unknown. An additional challenge lies in the use of model systems that are capable of reflecting the complexity of organisms and in the choice of methodology that is sensitive enough to capture the gradual and dynamic changes of HSFs. Investigations on common human diseases, especially cancer and neurodegeneration, have provided evidence for how dysregulated stress signaling networks are embedded within pathological processes. In-depth characterization of the regulatory components in such stress signaling networks is instrumental for identifying novel therapeutic opportunities. Future studies will reveal the molecular mechanisms underlying the intersection of cellular stress responses and disruption of proteostasis in pathologies.

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The authors declare no competing interests.

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