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STATE-OF-THE-ART REVIEW

Interplay between mammalian heat shock factors 1 and 2 in physiology and pathology

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The heat-shock factors (HSFs) belong to an evolutionary conserved family of transcription factors that were discovered already over 30 years ago. The HSFs have been shown to a have a broad repertoire of target genes, and they also have crucial functions during normal development. Importantly, HSFs have been linked to several disease states, such as neurodegenerative disorders and cancer, highlighting their importance in physiology and pathology. However, it is still unclear how HSFs are regulated and how they choose their specific target genes under different conditions. Posttranslational modifications and interplay among the HSF family members have been shown to be key regulatory mechanisms for these transcription factors. In this review, we focus on the mammalian HSF1 and HSF2, including their interplay, and provide an updated overview of the advances in understanding how HSFs are regulated and how they function in multiple processes of development, aging, and disease. We also discuss HSFs as therapeutic targets, especially the recently reported HSF1 inhibitors.

Introduction

Transcriptional regulation is crucial during the life of an organism. Transcription factors are regulatory proteins that bind to DNA and facilitate formation of multiprotein complexes at specific genomic sites to regulate gene expression, thereby coordinating the cellular response to diverse signals. In eukaryotes, transcription factors interact and function in a combinatorial manner, and they typically work as dimers [1]. Most transcription factors can form either homo- or heterodimers, by which the DNA-binding specificity and affinity can be regulated, thereby expanding their target site repertoire [2]. Many transcription factors

Abbreviations
17-AAG, 17-N-allylamino-17-demethoxygeldanamycin; AD, activation domain; ALS, amyotrophic lateral sclerosis; APC/C, anaphase-promoting complex/cyclosome; ATF1, cyclic AMP-dependent transcription factor; CAC, colitis-associated colon cancer; CAF, cancer-associated fibroblast; CaSig, cancer signature; ChIP-on-chip, chromatin immunoprecipitation followed by microarray hybridization; ChIP-seq, chromatin immunoprecipitation followed by sequencing; DBD, DNA-binding domain; DTHIB, direct targeted HSF1 inhibitor; ECM, extracellular matrix; eRNA, enhancer RNA; FBXW7, F-box and WD repeat domain containing 7; FDA, U. S. Food and Drug Administration; GBA, glucosidase β-acid; GlcNAcylation, *O*-linked β-*N*-acetylglucosamine modification; GSK-3β, glycogen synthase kinase 3 beta; HR-A/B, heptad repeat A/B; HR-C, heptad repeat C; HSE, heat-shock element; HSF, heat-shock factor; HSP, heat-shock protein; IFN-γ, interferon-γ; IL-6, interleukin 6; INHBA, inhibin subunit beta A; LIMIT, lncRNAinducing MHC-I and immunogenicity of tumor; MHC-I, major histocompatibility complex I; MLL, myeloid/lymphoid or mixed-lineage leukemia; NEDD4, neuronal precursor cell-expressed developmentally downregulated 4; nSB, nuclear stress body; PD-L1, programmed death-ligand 1; PIC, preinitiation complex; PTM, posttranslational modification; RD, regulatory domain; TAX1BP1, Tax1-binding protein 1; THBS2, thrombospondin 2.

belong to families composed of multiple isoforms, with capacity of binding to similar DNA sequences, which makes the transcriptional regulation even more multifaceted [3].

The heat-shock factor (HSF) is an evolutionary conserved transcription factor that binds DNA as a trimer. Only one HSF is found in invertebrates, whereas the vertebrate family of HSFs consists of at least seven members; HSF1-5 and HSFX and HSFY [4]. Among the mammalian HSFs, HSF1 is functionally most similar to the sole invertebrate HSF, as it is essential for stress-induced heat-shock protein (HSP) production. This review focuses mainly on HSF1 and HSF2 that are rare transcription factors forming either homo- or heterotrimers in mammals (Table 1). Since the original discoveries of HSF1 as a key transcription factor during acute stress, HSFs are now recognized to regulate gene expression beyond HSPs and stress responses (for recent comprehensive reviews, [4,5]), and many new targets genes have been identified [6,7]. Importantly, HSFs are activated by both intrinsic and extrinsic signals during cell differentiation and developmental processes, such as gametogenesis and neurogenesis. Therefore, it is not surprising that HSFs are also involved in many pathologies, such as cancer and neurodegenerative diseases.

Multifaceted properties of HSFs

The function of a transcription factor is determined by its structural features. The HSF family members have functional domains that are either shared or unique (see more details in Fig. 1). All HSF family members contain an amino-terminal winged helix-turn-helix DNA-binding domain (DBD). Two recent crystallographic studies have solved the structure of HSF1 and HSF2 DBDs bound to DNA [8,9]. These studies showed that the carboxy-terminal part of the DBD wraps around the entire DNA double helix and positions the rest of the HSF protein on the other side of the DNA. The DNA-binding activity of HSFs requires trimerization. Mammalian HSFs trimerize upon activation via intermolecular interactions between the HR-A/B oligomerization domain which forms a triple coiled-coil between three HSF monomers [10]. The HR-C found in the carboxy terminus interacts intramolecularly with the HR-A/B and represses oligomerization under nonstressed conditions [11]. The largely unstructured transactivation domain (AD) is required for HSFs to induce transcription and enables interaction with other transcription factors, cofactors, and chromatin remodelers [12–16]. The regulatory domain (RD) in HSF1 has a heat-sensing capacity and is important for transactivation [17]. Although a putative RD has been identified in the C-terminal part of HSF2 (Fig. 1), it may have negative regulatory functions, that is, an opposite effect to that of HSF1 [14,18].

As is common in many transcription factor families, also HSF1 and HSF2 can bind to the same DNA sequences, called heat-shock elements (HSEs), in the genome (Fig. 2). However, there are also reports of HSEs that are occupied only by either HSF1 or HSF2, as exemplified by GBA (glucosidase β acid) for HSF1 and MLL (myeloid/lymphoid or mixed-lineage leukemia) or p35 (cyclin-dependent kinase 5 activating protein) for HSF2 [19–23]. The HSE was originally identified from only a small set of target genes and defined as inverted pentameric nGAAn repeats (n = any nucleotide), and at least three continuous inverted repeats of nGAAn were found in the HSP gene promoters [24]. Subsequently, the target gene repertoire has increased and the HSE architecture displays a great variation, such as spacing, orientation, and number of repeats, and the exact sequence of the cis-acting element can also vary [6,7,25]. The

Table 1. Shared and unique features of HSF1 and HSF2.

Feature	HSF1	HSF2	References
Transcriptional regulation	Not known	HSE in promoter where HSF1 can bind	[79]
lncRNA, miRNA	HSR1 lncRNA	miR-18, miR-144, TUG1 lncRNA	[52,136–138]
Stability	Stable	Unstable, half-life decreases in the absence of HSF1	[41,53,55,56]
Degradation; Examples of E3 ligases	Via ubiquitination; FBXW7, NEDD4	Via ubiquitination; APC/C	[41,43,44]
Cellular localization, as inactive	Cytosol and nucleus, monomer	Cytosol, dimer	[139,140]
Cellular localization, as active	Nucleus, trimer, either homo- or heterotrimer	Nucleus, trimer, either homo- or heterotrimer	[139,140]
Binding to nuclear stress bodies, nSBs	Yes	Yes	[51,141,142]
Interacting partners	Many	Few known	[135]
Extracellular	Not known	Not known	

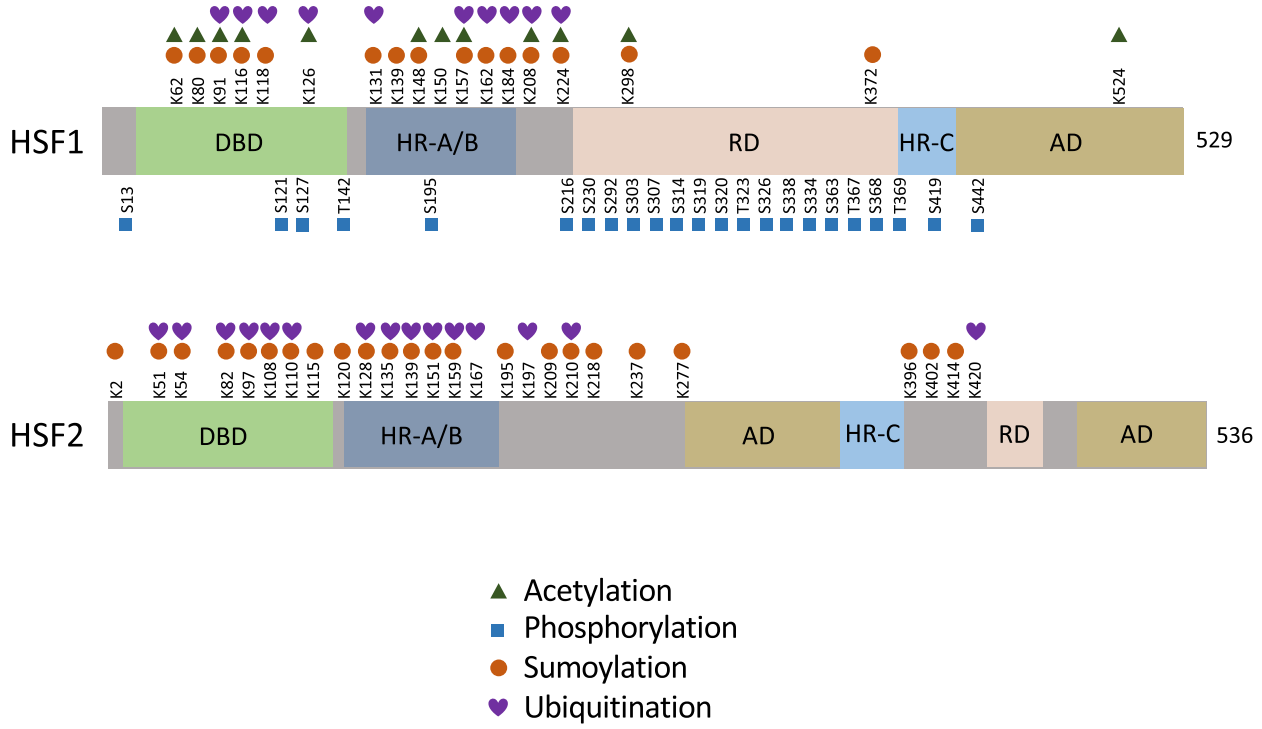


Fig. 1. Comparison of the functional domains of human HSF1 and HSF2, and posttranslational modifications therein. The DNA-binding domain (DBD) in HSF1 and HSF2 is largely conserved (70% identity, 80% similarity), whereas the rest of the protein is less conserved (approximately 35% identity). The trimerization domain contains two heptad repeats, HR-A and HR-B, forming a leucine zipper, and the C-terminal heptad repeat HR-C can interact with HR-A/B and repress trimerization. The regulatory domain RD is extensively phosphorylated in HSF1, but no PTMs have been reported within the RD of HSF2. The transactivation domain AD resides in the C terminus. The identified acetylation, phosphorylation, sumoylation, and ubiquitination sites are indicated on both HSF1 and HSF2. Some lysines may be conjugated by different chemical groups or whole proteins. It is important to note that not all sites are modified simultaneously. Note also that not all sumoylation and ubiquitination sites have been experimentally validated, as they have been identified in large-scale proteome-wide studies.

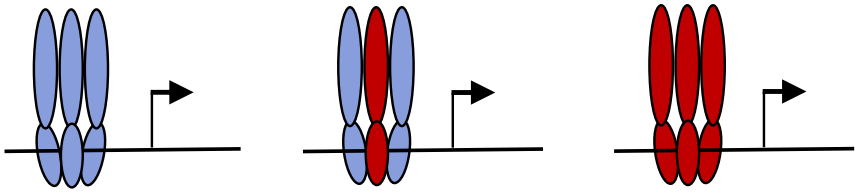
differences in the HSE architecture and within the chromatin landscape may therefore be important determinants for HSF binding to their specific target genes [19,26,27].

Transcription factors regulate gene expression not only by binding to promoters but also by binding and activating enhancers. Enhancers are distal regulatory sequences found upstream or downstream of genes, or even within genes, and they promote transcription of nearby genes by forming a loop between the enhancer and the gene promoter [28]. Enhancers can be transcribed to produce eRNAs, which can regulate both enhancer activity and transcriptional activity of nearby genes, but the underlying mechanisms are still largely unknown [29]. Genome-wide analyses have revealed that different forms of stress, for example, heat shock, induce both up- and downregulation of enhancers [19,30,31]. HSF1 has been shown to bind to a multitude of enhancers and transactivate genes by activating nearby enhancers in response to heat stress [19,31,32] (Fig. 2). Almost 500 enhancers are induced by heat shock in an HSF1-dependent manner. For example, in the Tax1 binding protein 1 (TAX1BP1) locus, HSF1 was found to bind only to a divergently transcribed enhancer 4.5 kb upstream of the gene promoter but it was essential for the heat-induced eRNA transcription and for the release of paused RNA polymerase from the TAX1BP1 promoter [32]. Further studies are required to determine whether also HSF1-HSF2 heterotrimers or HSF2 homotrimers can bind and regulate transcription via enhancers.

Posttranslational modifications regulate HSF1 and HSF2

Transcription factors are modified after their synthesis by conjugation of chemical groups (e.g., acetyl, methyl, and phosphate) or of whole proteins (e.g., SUMO and ubiquitin). These posttranslational modifications (PTMs) may regulate localization to the

A



Untreated cells	+	+	+	[6,43,136]
Acute stress	++	++	+	[6,43,136]
Mitosis	+	+	++	[6]
Development				
corticogenesis	-	+	+	[23] *
spermatogenesis	+	+	+	[20,21]
Neurodegenerative diseases	n.d.	n.d.	n.d.	
Cancer	+ +	n.d.	n.d.	[7]

B

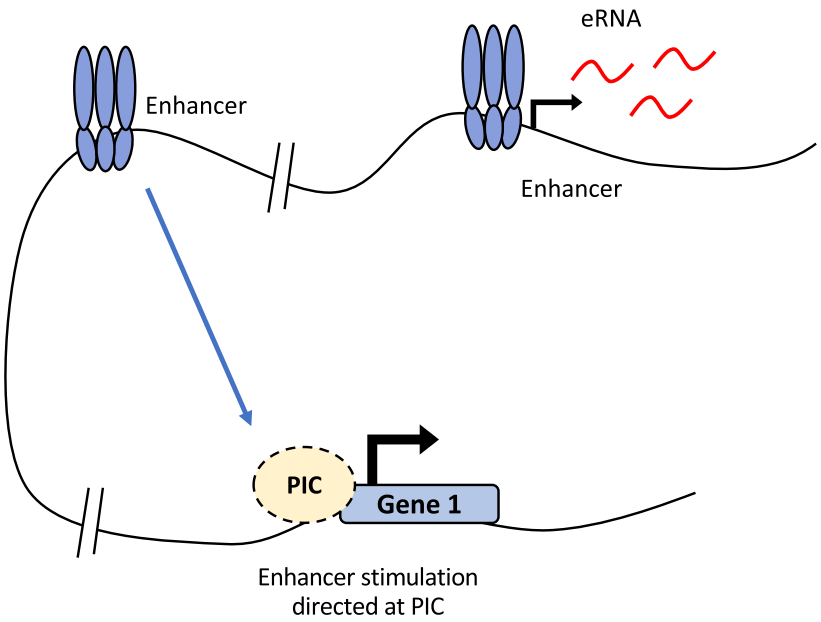


Fig. 2. Interaction of HSF1 and HSF2 at the chromatin. Panel A summarizes results from genome-wide ChIP-seq or ChIP-on-chip analyses of HSF1 (blue) and HSF2 (red) binding to their target genes, and panel B illustrates a newly discovered function of HSF1, transactivating genes via enhancers. (A) The conditions under which HSF1 or HSF2 DNA binding was performed are indicated to the left and the binding activity is assessed as: + basic levels, ++ increased levels. When both HSF1 and HSF2 were found to bind to the same gene, they are depicted as an HSF1-HSF2 heterotrimer. Note that the stoichiometry of HSF1 and HSF2 in the heterotrimer can vary, and heterotrimerization has not been studied on a genome-wide scale. To formally prove heterotrimerization, a sequential chromatin immunoprecipitation would be required, with one antibody against one HSF, followed by a second immunoprecipitation using the first pulldown material. As indicated in panel B, HSF1 can operate through enhancers, but the exact mechanisms are still not clear [19,31,32]. Enhancers can also be transcriptionally active and produce eRNAs. It should be emphasized that no comprehensive genome-wide analyses of HSF1-mediated eRNA-induced transcription are yet available. PIC stands for preinititation complex, n.d. for not determined. * study not genome-wide.

nucleus, DNA-binding, interaction with other factors, transactivation, attenuation, and degradation [33]. HSF1 and HSF2 undergo different PTMs, including acetylation, phosphorylation, sumoylation, and ubiquitination that can have either positive or negative regulatory effects (Fig. 1, [3,4] for comprehensive reviews). When bound to DNA, the HSF family members expose biochemically distinct surfaces, which

could be used for PTMs or protein–protein interactions to specifically regulate the activity of these transcription factors [8,9,34].

HSF1 is phosphorylated at serines and threonines throughout the protein, with many sites localized at the RD [4,35,36]. HSF1 is hyperphosphorylated by heat shock and other protein-damaging stresses, and this may precede its DNA binding and transactivation capacity [37,38]. Multiple kinases have been identified that phosphorylate HSF1 at specific sites, and these kinases are regulated by different stimuli, such as stress-induced GlcNAcylation (*O*-linked β-*N*-acetylglucosamine modification) of GSK-3β [4,39]. Some HSF1 hyperphosphorylation sites have been connected to the transcriptionally active HSF1, especially S326 residing in the RD that is used as a proxy for HSF1 activation [4,35]. Intriguingly, disrupting all known HSF1 phosphorylation sites in the RD did not render HSF1 transcriptionally incompetent, as this mutant HSF1 was still able to localize to the nucleus and transactivate *HSP* genes [40]. Instead, the mutant had a lower threshold for activation than wild-type HSF1, suggesting that HSF1 RD phosphorylation provides with a fine-tuning mechanism for HSF1's activity [40]. Nevertheless, distinct phosphorylation events may be important in different disease states, regulating HSF1 activity and stability [4].

Both HSF1 and HSF2 proteins can be regulated by ubiquitin-mediated proteasomal degradation [41–45] (Table 1). Interestingly, the HSF1 protein levels are dysregulated in certain pathologies; for example, in neurodegenerative diseases, the amount of HSF1 is diminished, leading to impaired HSF1 target gene expression [44–47]. Aberrant HSF1 degradation by the ubiquitin E3 ligase NEDD4 underlies α-synucleinopathy [44]. Acetylation of K80 in the HSF1 DBD correlates with increased HSF1 ubiquitination [44]. HSF1 acetylation has multiple regulatory effects including stabilization of HSF1, and acetylation of K80 and K118 in the DBD of HSF1 during the attenuation phase of an acute heat shock inhibits HSF1 DNA-binding activity [42,48]. In cancer and Huntington's disease models, the ubiquitin ligase E3 FBXW7 promotes the degradation of HSF1 in a phosphorylation-dependent manner [43,45]. The two serine residues S303 and S307 need to be phosphorylated for FBXW7 and HSF1 to interact and for HSF1 to be degraded. In recent proteome-wide studies, several HSF1 ubiquitination sites have been identified [49,50], but their functional impact has not yet been validated (Fig. 1).

Quantitative changes in HSF2 levels have been observed during cell differentiation, stress, and disease [41,51–54]. In contrast to HSF1, which is a stable protein under acute stress, the amount of HSF2 decreases during heat stress, and importantly, in cells lacking HSF1, there is usually less HSF2 protein than in wild-type cells [55,56]. In response to acute stress, HSF2 is ubiquitinated and degraded [41]. Although mass spectrometric analyses have identified multiple lysines on HSF2 that can be ubiquitinated (Fig. 1), the sites have not been experimentally validated [49,50,57,58]. The anaphase-promoting complex/cyclosome (APC/C) E3 ligase can mediate HSF2 degradation, but it is plausible that other ubiquitin E3 ligases are involved in regulating HSF2 levels [41]. In mitosis, HSF2 protein and mRNA levels decline in multiple cell lines [59]. This affects the chromatin environment of the *HSPA1A* (*HSP70*) promoter, allowing HSF1 binding, which protects mitotic cells against acute heat stress [59]. The functional relevance of HSF2 degradation and the underlying mechanisms are still largely unknown.

Proteome-wide studies have shown that heat shock induces massive sumoylation of many transcription factors, including HSF1 and HSF2 [60–63] (Fig. 1). HSF1 is stress-inducibly sumoylated at K298 (a major site) and K126 (a minor site). Surprisingly, this sumoylation event is not required for the induction of the heat-shock response, but it rather suppresses HSF1 transactivation capacity [64–66]. A recent study showed, using purified proteins, that trimerized HSF1 is more efficiently sumoylated at K298 than monomeric HSF1, but sumoylation does not interfere with HSF1 DNA-binding or HSC70-mediated dissociation from DNA *in vitro* [67]. In contrast, sumoylation of HSF2 at K82 in the DBD inhibits its DNA-binding activity [68–71]. Multiple sumoylation sites on both HSF1 and HSF2 in the DBD and HR-A/B have been identified in an independent mass spectrometric analysis using cell lines exposed to proteotoxic stresses [72]. Although the functional effects of these modifications have not been established, it is likely that sumoylation may have a more multifaceted role in regulating trimerization and DNA binding of HSFs than previously anticipated.

Taken together, the complex effects of different PTMs on HSFs, where some sites may be modified by multiple PTMs or where one PTM depends on another, provide with a fine-tuning mechanism for controlling the activity of the HSFs. A major challenge lies in characterizing the specific PTMs and their function in various cell types and tissues under physiological and pathological conditions.

Trimerization of HSF1 and HSF2 modulates transcriptional capacity

HSF1 and HSF2 are co-expressed in many tissues, and they can form heterotrimers, suggesting an interplay

between HSF1 and HSF2 [51,73]. Genome-wide ChIP-on-chip and ChIP-seq analyses have demonstrated that HSF1 and HSF2 can bind to the same target gene promoters [6,20–22] (Fig. 2). However, from these studies it is impossible to distinguish if HSFs bind as heterotrimers or homotrimers. Certain genes are also found to be specific for either HSF1 or HSF2. Cells devoid of HSF1 cannot activate a heat-shock response, showing that HSF2 is not able to compensate for the loss of HSF1 [74]. Nevertheless, heterotrimerization of HSF1-HSF2 may modulate the expression of certain genes, since cells lacking HSF2 induce HSPs to a lower level than wild-type cells [56]. HSF2 is considered responsive to chronic stress, for example at fever-like temperatures (39–41 °C) and upon exposures to ethanol or proteasome inhibition [73,75–78]. The proteasome inhibitor bortezomib causes an increase in both mRNA and protein levels of HSF2 in human primary cells [73]. Recently, a new interrelationship between HSF1 and HSF2 was suggested when HSF1 was shown to transcriptionally regulate the levels of HSF2 by binding to an HSE in the *HSF2* gene promoter in cells subjected to proteasome inhibition [79].

HSFs in development, aging, and degeneration

The HSFs are an exceptional class of transcription factors. In addition to responding rapidly to different forms of protein-damaging stresses throughout the life of an organism, they are also vital for developmental and differentiation-related processes. To study the interdependency between HSFs in vertebrates, model organisms with multiple HSF family members, such as zebrafish and mice, have been used. Although mice deficient of HSF1, HSF2, or HSF4 are viable, they all display different phenotypes, suggesting that individual HSFs have their specific functions during development [74,80,81]. For example, $HSF1^{-/-}$ mice display a reduced body and organ size, which may be due to compromised protein synthesis [81]. Interestingly, there is a synergy between HSF1 and HSF2, as both $HSF1^{-/-}$ and $HSF2^{-/-}$ mice have impaired gametogenesis, whereas the double knockout male mice are completely sterile [20,21,52,81–84]. The crosstalk between HSF1 and HSF2 in development is demonstrated by genome-wide studies, revealing that HSF1 and HSF2 can bind to the same genomic regions in spermatogenic cells [20].

HSF1 and HSF2 are important for brain development and function by regulating crucial processes, such as neuronal migration, neuronal synapse formation, and responses to proteotoxic stress [85–88] (for a recent review, [89]). These HSFs interact and play dual roles in brain development and stress response, which has been demonstrated using mice exposed to prenatal alcohol treatment [76]. HSF2, which under normal conditions forms homotrimers at certain target genes and promotes neural migration, heterotrimerized with HSF1 when mice were treated with ethanol. The HSF1-HSF2 heterotrimerization alters the gene expression program by inducing transcription of HSPs to promote cell survival upon ethanol exposure. Intriguingly, this prenatal stress leads to neural migration defects as neuro-specific HSF2 target genes are not expressed anymore. HSFs may therefore play a role in neuropsychiatric disorders of neurodevelopmental origin due to prenatal insults [90].

HSF1 is required for larval development of the nematode *C. elegans* [91,92]. A recent study uncovered how stress signals in neurons can be transmitted to vulnerable germ cells to provide protection against damage [93]. In *C. elegans*, serotonin is released by maternal neurons during stress, which ensures both the viability and stress resilience of future offspring. Serotonin acts through a signal transduction pathway which is conserved between *C. elegans* and mammalian cells [93]. In mammalian neuronal cells, serotonin increases *HSP* mRNA and protein levels in an HSF1-dependent manner. In soon to be fertilized germ cells, serotonin enables HSF1 to alter the chromatin landscape by recruiting the histone chaperone FACT, which results in displacement of histones, thereby allowing active transcription of protective genes such as *HSPs* [93]. Further studies regarding HSF1 activation by the neurotransmitter serotonin in human cells are warranted, especially if increased HSF1 activation could be utilized for developing therapy for neurodegenerative diseases.

During aging, there is a deterioration in the ability to properly respond to external cues, which coincides with a failure in maintaining proteostasis, that is, the balance between protein synthesis, folding, and degradation [94]. This aging-related proteostasis collapse has been well established in nematodes, whereas it has been unclear if a similar phenomenon exists in mammals [95,96]. A recent study shows, using transcriptome-wide characterization of gene expression, splicing, and translation, that significant deterioration occurs in the transcriptional activation of the heat shock response in stressed human senescent cells [97]. Mechanistically, both the nuclear localization and subnuclear distribution of HSF1 phosphorylated at S326, a marker for activated HSF1, are impaired. The proteasome function also declines in stressed senescent cells, and it is not recovered when the cells are placed

back to normal temperature [97]. The proteostasis collapse may have multiple implications on how humans age.

The prevalence of human neurodegenerative diseases increases with age [36]. The hallmarks of many neurodegenerative diseases are accumulation of misfolded proteins and a proteostasis collapse due to imbalance in protein folding and degradation. HSF1 has a protective role and also genes other than HSPs are affected [98]. In contrast to HSF1, it is still unknown whether HSF2 is involved in neurodegenerative diseases. Nevertheless, an *HSF2*$^{-/-}$ mouse model for Huntington's disease displays increased protein aggregation in the brain and a reduced life span, suggesting that HSF2 may have a role in regulating neuronal proteostasis. Although the underlying molecular mechanisms for HSF2 function are unclear, accumulation of protein aggregates in the absence of HSF2 is partially due to changes in chaperone levels, especially αB-crystallin expression [75]. HSF2 has also been identified as a gene whose expression is commonly downregulated during aging when performing a meta-analysis of transcriptional changes associated with Alzheimer's disease and aging [99].

HSF1 and HSF2 in cancer

In numerous types of cancer, elevated chaperone levels driven by HSF1 have been observed, which correlates with poor prognosis, increased metastatic potential, and resistance to therapy [100]. A key role for HSF1 in cancer was established when *HSF1*$^{-/-}$ mice were found to be resistant to tumorigenesis [101,102]. In human tumors, HSF1 protein levels are frequently elevated, mainly due to gene amplification, or due to mutations in the ubiquitin E3 ligase FBXW7, which supports tumorigenesis [43,103]. FBXW7 is a nuclear protein, frequently mutated in multiple different cancers [104], suggesting that FBXW7 controls the stability of nuclear HSF1 in cancer. Interestingly, only a few HSF1 mutations have been found in cancers, suggesting that HSF1 is needed intact for transformation and tumor progression [105]. In many cancer types, HSF1 is found active in the nucleus, and consequently, HSF1 levels and nuclear localization correlate with the degree of malignancy. Since increased HSF1 protein expression corresponds with the progress of prostate and breast cancer, and poor disease-specific survival, nuclear HSF1 could be used as a prognostic marker [106,107].

HSF1 drives oncogenesis by regulating a specific cancer gene expression signature (HSF1 CaSig), composed of 456 genes, which includes not only genes encoding chaperones but also genes involved in cell adhesion, cell cycle control, metabolism, proliferation, protein translation, and signaling [7]. This gene expression profile is different from that induced by HSF1 during heat shock. Importantly, the HSF1 CaSig could be used for therapeutic and prognostic applications, and retrospective studies have shown that melanoma patients with high expression of HSF1 and CaSig genes have poorer outcome and overall survival than patients with less HSF1 [7,103,108].

HSF1 has been implicated in immunological responses as interleukin 6 (IL-6) has been shown to be a direct target gene for HSF1, and certain HSF1 CaSig genes are involved in immune functions [7,109]. In breast cancer cells, programmed death-ligand 1 (PD-L1), a target for cancer immunotherapy, is an HSF1 target gene, and phosphorylation of HSF1 at T120 induces higher expression of PD-L1 [110]. Recently, a role for HSF1 in antitumor immunity was reported, as HSF1 can regulate interferon γ (IFNγ)-induced major histocompatibility complex I (MHC-I) expression [111]. In this study, a novel immunogenic long noncoding RNA, *LIMIT*, was shown to *cis*-activate a guanylate-binding protein (GBP) gene cluster, leading to disrupted HSP90-HSF1 interaction and HSF1 activation. Interestingly, the authors also suggest that the LIMIT-GBP-HSF1 axis could be targetable for immunotherapy.

Malignant cells within a tumor are surrounded by many different types of cells, such as fibroblasts, immune, and endothelial cells as well as components of extracellular matrix (ECM). This microenvironment is essential for tumor formation and progression [112]. Cancer-associated fibroblasts (CAFs) support cancer cells in a noncell autonomous manner by secretion of ECM, chemokines, cytokines, and growth factors. HSF1 is frequently activated in CAFs where it drives a transcriptional program enabling malignancy. The transcriptional program is clearly different from that driven by HSF1 in adjacent cancer cells [113]. In fibroblasts that are cocultured with cancer cells, HSF1 regulates the expression of genes involved in cell adhesion and wound healing, which activates ECM genes in adjacent cancer cells [113,114]. Recently, it was shown that HSF1 is crucial in the remodeling of the ECM structure and composition in a mouse model of colitis-associated colon cancer (CAC) [115]. Loss of HSF1 abrogates ECM assembly by colon fibroblasts, prevents inflammation-induced ECM remodeling, and inhibits progression to CAC in mice. These findings are recapitulated in CAC patients, where a strong activation of stromal HSF1 is accompanied with a high expression of many ECM-affiliated HSF1 target genes [115]. HSF1 also regulates a transcriptional profile that

promotes malignancy in CAFs from gastric cancer patients [116]. HSF1 upregulates inhibin subunit beta A (INHBA) and thrombospondin 2 (THBS2), and these proteins are secreted in CAF-derived extracellular vesicles to the tumor microenvironment to promote cancer [116]. Accumulating evidence indicates that HSF1 functions as a master regulator in CAFs in multiple carcinomas from different tissues and that release of extracellular vesicles is one way to promote tumorigenesis of nearby cells.

So far, there are only a few reports on involvement of HSF2 in cancer [54,117], which was highlighted in a recent comprehensive review [118]. HSF2 expression is frequently decreased in several human malignancies, and the decreased HSF2 expression correlates with the aggressiveness in clinical samples of prostate cancer [54]. In contrast, in hepatocellular cancer, patients with higher levels of HSF2 display worse survival [117]. A loss of HSF2 increases tumor growth and invasive properties, and silencing HSF2 in prostate cancer cells promotes invasion through altered expression of genes linked to focal adhesion and actin cytoskeleton [54]. HSF2 is also implicated in proteasome inhibitor-mediated control of cancer cell migration [79]. A recent study in human osteosarcoma cells revealed the importance of HSF2 in maintaining cell–cell adhesion during proteotoxic stress [119]. HSF2 is indispensable for cell survival after prolonged proteasome inhibition, and the ability to survive proteotoxic stress is not only dependent on induction of chaperones, but involves multiple targets in different pathways, including genes belonging to the cadherin superfamily. Interestingly, studies in human cells or mouse testis have also identified cadherins as HSF2 target genes [6,20]. Additional studies are needed to understand the role of HSF2 in cell–cell adhesion and to study HSF2 in CAFs. It would be particularly important to identify the HSF2-driven cancer-specific gene expression signature in different types of cancer.

Pharmacological regulation of HSFs

HSF1 inhibitors

Because many cancer cells are clearly dependent on HSF1 due to a so-called nononcogene addiction, and HSF1 levels or the HSF1 transcriptional signature correlate with the survival of cancer patients, pharmacological targeting of HSF1 is desirable [103,120]. However, there are major challenges with the druggability of transcription factors, such as HSF1, which has no enzymatic activity and contains structurally extended regions of disorder. Nevertheless, several HSF1 inhibitors have been identified based on natural product library screens and different screening methodologies [103]. In most cases, the compounds have been found utilizing HSE-promotor reporter assays, and they may impact either basal or stress-induced activity. Using this approach, many compounds identified may not be direct HSF1 inhibitors, but instead inhibit general transcription, translation, or upstream signaling pathways, and they may also have 'off-target' effects [103].

In addition to natural molecules, some synthetic compounds have emerged as potential HSF1 inhibitors. For example, KRIBB11 was found in an HSE-luciferase reporter assay, and it was shown to coprecipitate with HSF1 [121]. However, KRIBB11 may also accelerate Mcl-1 degradation through an HSF1-independent pathway, suggesting that KRIBB11 is not specific for HSF1 [122]. Another synthetic compound, $I_{HSF}115$, developed using in silico screening, binds both the full-length HSF1 and the HSF1 DBD in a surface plasmon resonance analysis [123]. $I_{HSF}115$ can disrupt the interaction between HSF1 and ATF1. ATF1 recruits transcriptional coregulators and modulates HSF1 activity by maintaining an open chromatin state at HSF1 target loci. Although many functional properties of HSF1, such as the DNA-binding, protein stability, and trimerization, are not affected by $I_{HSF}115$, it is cytotoxic to a variety of cancer cell lines [123].

Recently, a novel type of HSF1 inhibitor, named DTHIB, was developed by screening for small molecules binding to the structurally well-ordered HSF1 DBD using differential scanning fluorimetry [124]. This study demonstrated that DTHIB is a direct HSF1 inhibitor binding with high affinity to HSF1. DTHIB destabilized HSF1 protein levels especially in the nucleus, whereas cytosolic HSF1 was unaffected. Nuclear HSF1 was degraded by the proteasome in an FBXW7 ubiquitin E3 ligase-dependent manner. Using cell lines as well as mouse xenograft and syngeneic models, DTHIB was shown to inhibit therapy-resistant prostate cancer cell proliferation. Since DTHIB treatment broadly inhibited the HSF1 CaSig and HSF1-mediated transcriptional network [124], it will be interesting to see whether this inhibitor is efficacious toward other cancer types besides prostate cancer. DTHIB is a welcome therapeutic outcome of drug development, and it provides new possibilities to address outstanding mechanistic questions regarding HSFs in stress biology. To this end, the fate of HSF2 remains unclear when nuclear HSF1 is degraded, as HSF2 has been earlier shown to be less stable when HSF1 is downregulated or completely absent [55,56].

HSF1 activators

Activation of HSF1 has been considered as a therapeutic approach against neurodegenerative diseases that are caused by protein misfolding, for example, Alzheimer's, Parkinson's, ALS (amyotrophic lateral sclerosis), and polyglutamine diseases including Huntington's disease. Multiple small compounds have been identified to activate endogenous HSF1 and induce HSP protein production, and some of them could be promising candidates in pharmacological treatment of neurodegenerative diseases [125]. However, most of these compounds are not direct HSF1 binders; instead, they bind and inhibit chaperones, such as HSP70, HSP90, or TriC, all proteins known to interact with HSF1 [126]. Importantly, HSF1 levels are considerably decreased in Huntington's and other neurodegenerative disease models, suggesting that compounds inhibiting HSF1 degradation and increasing HSF1 levels are required rather than those stimulating the transactivating capacity of HSF1 [44,45].

HSP90 inhibitors induce a heat-shock response

HSP90 is an essential and very abundant molecular chaperone in eukaryotic cells. HSP90 is a dimeric protein with a large repertoire of so-called client proteins, including kinases, phosphatases, growth factor receptors, and nuclear hormone receptors [127]. HSP90 can regulate both the function and stability of its client proteins. Since many HSP90 clients have crucial roles in rapidly growing cancer cells, inhibition of HSP90 suppresses many signaling pathways that are important for cancer. Thus, multiple HSP90 inhibitors have been developed for cancer therapies, and many of them target the N terminus of HSP90 which contains the ATP-binding site. However, despite several promising preclinical studies, not a single HSP90 inhibitor has been approved for a clinical use by the FDA [128]. Most HSP90 inhibitors induce a heat-shock response and trigger the transcriptional activity of HSF1 [129]. The increased levels of HSPs and HSF1 activation are unfortunately counterproductive when treating cancer. In addition, high doses of HSP90 inhibitors may have immunosuppressive functions. It has therefore been proposed that HSP90 inhibitors could be used at continuous low doses that do not induce a heat-shock response, in combination with other cancer drugs, to receive a better therapeutic efficacy [130,131].

To this date, it is unclear how N-terminal HSP90 inhibitors activate the heat-shock response. The current model proposes that HSF1 can be kept in an inactive state with HSP90, HSP70, and other chaperones, and that once HSP90 is inactivated, HSF1 is released from the complex, which allows its trimerization, and subsequent transcription of HSPs [132]. The knowledge regarding HSF2, HSP90, and HSP90 inhibitors is scarce. Intriguingly, a recent study shows that HSF2 can be coprecipitated with HSP90 when using specific conformationally strained, 'closed-form', HSP90 mutants [133]. Similarly to HSF1-HSP90 interaction, different HSP90 inhibitors are able to disrupt HSF2-HSP90 interaction [132,133]. Other studies have observed that the HSP90 inhibitor STA-9090 increases HSF2 levels in multiple bladder cancer cell lines [134]. In addition, *HSF2*$^{-/-}$ cells are more sensitive to HSP90 inhibitors geldanamycin and 17-AAG than wild-type cells [119]. Therefore, HSF2 levels and stability of HSF2 should be taken into consideration when studying HSP90 inhibitors.

Perspectives

During the past years, we have acquired increased knowledge of the expression patterns and functions of HSF1 and HSF2 in physiology and pathology. Many fundamental questions are still unanswered and are summarized in Box 1.

Although much less research has been performed on HSF2 than HSF1, there is accumulating evidence demonstrating a role for HSF2 in biological processes besides gametogenesis and corticogenesis. Interrelationship of HSF1 and HSF2 is still poorly understood, particularly whether they compete or work in synergy, and how the interplay changes depending on the cellular state (normal, stress, or disease state). So far, most studies addressing HSF interdependency have been performed in various cell lines exposed to acute stress, especially heat shock, and future studies directed

BOX 1. Outstanding questions

- Can HSF2 be transcriptionally active independently of HSF1 or other transcription factors?
- What is the HSF2 transcriptional signature?
- What changes HSFs from inducing one transcriptional program to another?
- What is the fate of HSF2 (and the other HSFs) when HSF1 is inhibited by novel inhibitors?
- Are there compensatory mechanisms when one or several members of the HSF family are deleted or downregulated (silenced)?

toward pathologies are required. Importantly, when developing and analyzing pharmacological regulators for HSF1 or the heat-shock response, HSF2 and other HSF family members should be monitored.

There are gaps in understanding how HSFs are regulated. HSF protein levels must be carefully tuned and PTMs may regulate both the stability of these proteins and their transcriptional activity. We still do not know how the formation of heterotrimers is regulated. Examples of questions to be addressed include the following: Do the different PTMs play a key role, or is it the ratio between HSF1 and HSF2 that determines heterotrimerization? Do HSF1 and HSF2 form complexes with other HSF family members, such as HSF4? Identification of proteins that interact with HSFs may give insights into how they are regulated and how the condition-specific target genes are selected. For this purpose, the HSF1 interactome during different disease conditions was recently reported, and HSF1 was shown to interact with a diverse group of proteins, and interestingly, more partner proteins were detected under stress than control situations [135].

The impact of HSFs in cancer is more complex than previously anticipated, since there is now clear evidence that HSF1 activity in CAFs promotes tumorigenesis and metastasis [113–116]. Extracellular vesicles derived from CAFs contain proteins encoded by HSF1 target genes that are critical for tumor progression [116]. This crosstalk emphasizes the importance of including both the stroma and cancer cells in forthcoming studies. In addition to different forms of cancer, many neurodegenerative diseases progress during aging, and abnormal functions of HSFs, particularly those of HSF1, have been implicated in aged cells and organisms. Thus, it remains to be established whether HSFs are dysregulated also in other aging-related diseases, including metabolic disorders.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

PRM and LS wrote the manuscript.

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