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Heat shock factors: integrators of cell stress, development and lifespan

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Abstract

Heat shock factors (HSFs) are essential for all organisms to survive exposures to acute stress. They are best known as inducible transcriptional regulators of genes encoding molecular chaperones and other stress proteins. Four members of the HSF family are also important for normal development and lifespan-enhancing pathways, and the repertoire of HSF targets has thus expanded well beyond the heat shock genes. These unexpected observations have uncovered complex layers of post-translational regulation of HSFs that integrate the metabolic state of the cell with stress biology, and in doing so control fundamental aspects of the health of the proteome and ageing.

In the early 1960s, Ritossa made the seminal discovery of temperature-induced puffs in polytene chromosomes of *Drosophila melanogaster* larvae salivary glands[1]. A decade later, it was shown that the puffing pattern corresponded to a robust activation of genes encoding the heat shock proteins (HSPs), which function as molecular chaperones[2]. The heat shock response is a highly conserved mechanism in all organisms from yeast to humans that is induced by extreme proteotoxic insults such as heat, oxidative stress, heavy metals, toxins and bacterial infections. The conservation among different eukaryotes suggests that the heat shock response is essential for survival in a stressful environment.

The heat shock response is mediated at the transcriptional level by *cis*-acting sequences called heat shock elements (HSEs; BOX 1) that are present in multiple copies upstream of the HSP genes[3]. The first evidence for a specific transcriptional regulator, the heat shock factor (HSF) that can bind to the HSEs and induce HSP gene expression, was obtained through DNA–protein interaction studies on nuclei isolated from *D. melanogaster* cells[4,5]. Subsequent studies showed that, in contrast to a single HSF in invertebrates, multiple HSFs are expressed in plants and vertebrates[6–8]. The mammalian HSF family consists of four members: HSF1, HSF2, HSF3 and HSF4. Distinct HSFs possess unique and overlapping functions (FIG. 1), exhibit tissue-specific patterns of expression and have multiple post-translational modifications (PTMs) and interacting protein partners[7,9,10]. Functional crosstalk between HSF family members and PTMs facilitates the fine-tuning of HSF-mediated gene regulation. The identification of many targets has further extended the impact

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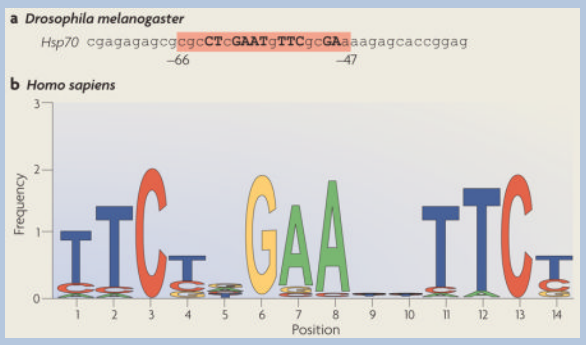
of HSFs beyond the heat shock response. Here, we present the recent discoveries of novel target genes and physiological functions of HSFs, which have changed the view that HSFs act solely in the heat shock response. Based on the current knowledge of small-molecule activators and inhibitors of HSFs, we also highlight the potential for pharmacologic modulation of HSF-mediated gene regulation.

Box 1

The heat shock element



Heat shock factors (HSFs) act through a regulatory upstream promoter element, called the heat shock element (HSE). In the DNA-bound form of a HSF, each DNA-binding domain (DBD) recognizes the HSE in the major groove of the double helix[6]. The HSE was originally identified using S1 mapping of transcripts of the *Drosophila melanogaster* heat shock protein (HSP) genes[3] (see the figure; part **a**). Residues –47 to –66 are necessary for heat inducibility. HSEs in HSP gene promoters are highly conserved and consist of inverted repeats of the pentameric sequence nGAAn[132]. The type of HSEs that can be found in the proximal promoter regions of HSP genes is composed of at least three contiguous inverted repeats: nTTCnnGAAnnTTCn[132–134]. The promoters of HSF target genes can also contain more than one HSE, thereby allowing the simultaneous binding of multiple HSFs. The binding of an HSF to an HSE occurs in a cooperative manner, whereby binding of an HSF trimer facilitates binding of the next one[135]. More recently, Trinklein and colleagues used chromatin immunoprecipitation to enrich sequences bound by HSF1 in heat-shocked human cells to define the HSE consensus sequence. They confirmed the original finding of Xiao and Lis, who identified guanines as the most conserved nucleotides in HSEs[87,133] (see the figure; part **b**). Moreover, in a pair of inverted repeats, a TTC triplet 5′ of a GAA triplet is separated by a pyrimidine–purine dinucleotide, whereas the two nucleotides separating a GAA triplet 5′ from a TTC triplet is unconstrained[87]. The discovery of novel HSF target genes that are not involved in the heat shock response has rendered it possible that there may be HSEs in many genes other than the HSP genes. Although there are variations in these HSEs, the spacing and position of the guanines are invariable[7]. Therefore, both the nucleotides and the exact spacing of the repeated units are considered as key determinants for recognition by HSFs and transcriptional activation. Part **b** of the figure is modified, with permission, from REF. 87 © (2004) The American Society for Cell Biology.

HSFs as stress integrators

A hallmark of stressed cells and organisms is the increased synthesis of HSPs, which function as molecular chaperones to prevent protein misfolding and aggregation to maintain protein homeostasis, also called proteostasis[11]. The transcriptional activation of HSP genes is mediated by HSFs (FIG. 2a), of which HSF1 is the master regulator in vertebrates. *Hsf1*-

knockout mouse and cell models have revealed that HSF1 is a prerequisite for the transactivation of HSP genes, maintenance of cellular integrity during stress and development of thermotolerance[12–15]. HSF1 is constitutively expressed in most tissues and cell types[16], where it is kept inactive in the absence of stress stimuli. Thus, the DNA-binding and transactivation capacity of HSF1 are coordinately regulated through multiple PTMs, protein–protein interactions and subcellular localization. HSF1 also has an intrinsic stress-sensing capacity, as both *D. melanogaster* and mammalian HSF1 can be converted from a monomer to a homotrimer *in vitro* in response to thermal or oxidative stress[17–19].

Functional domains

HSFs, like other transcription factors, are composed of functional domains. These have been most thoroughly characterized for HSF1 and are schematically presented in FIG. 2b. The DNA-binding domain (DBD) is the best preserved domain in evolution and belongs to the family of winged helix-turn-helix DBDs[20–22]. The DBD forms a compact globular structure, except for a flexible wing or loop that is located between β-strands 3 and 4 (REF. 6). This loop generates a protein– protein interface between adjacent subunits of the HSF trimer that enhances high-affinity binding to DNA by cooperativity between different HSFs[23]. The DBD can also mediate interactions with other factors to modulate the transactivating capacity of HSFs[24]. Consequently, the DBD is considered as the signature domain of HSFs for target-gene recognition.

The trimerization of HSFs is mediated by arrays of hydrophobic heptad repeats (HR-A and HR-B) that form a coiled coil, which is characteristic for many Leu zippers[6,25] (FIG. 2b). The trimeric assembly is unusual, as Leu zippers typically facilitate the formation of homodimers or heterodimers. Suppression of spontaneous HSF trimerization is mediated by yet another hydrophobic repeat, HR-C[26–28]. Human HSF4 lacks the HR-C, which could explain its constitutive trimerization and DNA-binding activity[29]. Positioned at the extreme carboxyl terminus of HSFs is the transactivation domain, which is shared among all HSFs[6] except for yeast Hsf, which has transactivation domains in both the amino and C termini, and HSF4A, which completely lacks a transactivation domain[29–31]. In HSF1, the transactivation domain is composed of two modules — AD1 and AD2, which are rich in hydrophobic and acidic residues (FIG. 3a) — that together ensures a rapid and prolonged response to stress[32,33]. The transactivation domain was originally proposed to provide stress inducibility to HSF1 (REFS 34,35), but it soon became evident that an intact regulatory domain, located between the HR-A and HR-B and the transactivation domain, is essential for the responsiveness to stress stimuli[32,33,36,37]. Because several amino acids that are known targets for different PTMs reside in the regulatory domain[33,38–42], the structure and function of this domain are under intensive investigation.

Regulation of the HSF1 activation–attenuation cycle

The conversion of the inactive monomeric HSF1 to high-affinity DNA-binding trimers is the initial step in the multistep activation process and is a common feature of all eukaryotic HSFs[43,44] (FIG. 3b). There is compelling evidence for HSF1 interacting with multiple HSPs at different phases of its activation cycle. For example, monomeric HSF1 interacts weakly with HSP90 and, on stress, HSF1 dissociates from the complex, allowing HSF1 trimerization[45,46] (FIG. 3b). Trimeric HSF1 can be kept inactive when its regulatory domain is bound by a multi-chaperone complex of HSP90, co-chaperone p23 (also known as PTGES3) and immunophilin FK506-binding protein 5 (FKBP52; also known as FKBP4)[46–51]. Elevated levels of both HSP90 and HSP70 negatively regulate HSF1 and prevent trimer formation on heat shock[52]. Activated HSF1 trimers also interact with HSP70 and the co-chaperone HSP40 (also known as DNAJB1), but instead of suppressing the DNA-binding activity of HSF1, this interaction inhibits its transactivation capacity[52–54].

Although the inhibitory mechanism is still unknown, the negative feedback from the end products of HSF1-dependent transcription (the HSPs) provides an important control step in adjusting the duration and intensity of HSF1 activation according to the levels of chaperones and presumably the levels of nascent and misfolded peptides.

A ribonucleoprotein complex containing eukaryotic elongation factor 1A (eEF1A) and a non-coding RNA, heat shock RNA-1 (HSR-1), has been reported to possess a thermosensing capacity. According to the proposed model, HSR-1 undergoes a conformational change in response to heat stress and together with eEF1A facilitates trimerization of HSF1 (REF. 55). How this activation mode relates to the other regulatory mechanisms associated with HSFs remains to be elucidated.

Throughout the activation–attenuation cycle, HSF1 undergoes extensive PTMs, including acetylation, phosphorylation and sumoylation (FIG. 3). HSF1 is also a phosphoprotein under non-stress conditions, and the results from mass spectrometry (MS) analyses combined with phosphopeptide mapping experiments indicate that at least 12 Ser residues are phosphorylated[41,56–59]. Among these sites, stress-inducible phosphorylation of Ser230 and Ser326 in the regulatory domain contributes to the transactivation function of HSF1 (REFS 38,41). Phosphorylation-mediated sumoylation on a single Lys residue in the regulatory domain occurs rapidly and transiently on exposure to heat shock; Ser303 needs to be phosphorylated before a small ubiquitin-related modifier (SUMO) can be conjugated to Lys298 (REF. 39). The extended consensus sequence ΨKxExxSP has been named the phosphorylation-dependent sumoylation motif (PDSM; FIG. 3)[40]. The PDSM was initially discovered in HSF1 and subsequently found in many other proteins, especially transcriptional regulators such as HSF4, GATA1, myocyte-specific enhancer factor 2A (MEF2A) and SP3, which are substrates for both SUMO conjugation and Pro-directed kinases[40,60–62].

Recently, Mohideen and colleagues showed that a conserved basic patch on the surface of the SUMO-conjugating enzyme ubiquitin carrier protein 9 (UBC9; also known as UBE2I) discriminates between the phosphorylated and non-phosphorylated PDSM of HSF1 (REF. 63). Future studies will be directed at elucidating the molecular mechanisms for dynamic phosphorylation and UBC9-dependent SUMO conjugation in response to stress stimuli and establishing the roles of kinases, phosphatases and desumoylating enzymes in the heat shock response. The kinetics of phosphorylation-dependent sumoylation of HSF1 correlates inversely with the severity of heat stress, and, as the transactivation capacity of HSF1 is impaired by sumoylation and this PTM is removed when maximal HSF1 activity is required[40], sumoylation could modulate HSF1 activity under moderate stress conditions. The mechanisms by which SUMO modification represses the transactivating capacity of HSF1, and the functional relationship of this PTM with other modifications that HSF1 is subjected to, will be investigated with endogenous substrate proteins.

Phosphorylation and sumoylation of HSF1 occur rapidly on heat shock, whereas the kinetics of acetylation are delayed and coincide with the attenuation phase of the HSF1 activation cycle. Stress-inducible acetylation of HSF1 is regulated by the balance of acetylation by p300–CBP (CREB-binding protein) and deacetylation by the NAD^+-dependent sirtuin, SIRT1. Increased expression and activity of SIRT1 enhances and prolongs the DNA-binding activity of HSF1 at the human *HSP70.1* promoter, whereas downregulation of SIRT1 enhances the acetylation of HSF1 and the attenuation of DNA-binding without affecting the formation of HSF1 trimers[42]. This finding led to the discovery of a novel regulatory mechanism of HSF1 activity, whereby SIRT1 maintains HSF1 in a state that is competent for DNA binding by counteracting acetylation (FIG. 3). In the light of current knowledge, the attenuation phase of the HSF1 cycle is regulated by a dual mechanism: a dependency on

the levels of HSPs that feed back directly by weak interactions with HSF1, and a parallel step that involves the SIRT1-dependent control of the DNA-binding activity of HSF1. Because SIRT1 has been implicated in caloric restriction and ageing, the age-dependent loss of SIRT1 and impaired HSF1 activity correlate with an impairment of the heat shock response and proteostasis in senescent cells, connecting the heat shock response to nutrition and ageing (see below).

HSF dynamics on the *HSP70* promoter

For decades, the binding of HSF to the *HSP70.1* gene has served as a model system for inducible transcription in eukaryotes. In *D. melanogaster*, HSF is constitutively nuclear and low levels of HSF are associated with the *HSP70* promoter before heat shock[64–66]. The uninduced *HSP70* promoter is primed for transcription by a transcriptionally engaged paused RNA polymerase II (RNAP II)[67,68]. RNAP II pausing is greatly enhanced by nucleosome formation *in vitro*, implying that chromatin remodelling is crucial for the release of paused RNAP II[69]. It has been proposed that distinct hydrophobic residues in the transactivation domain of human HSF1 can stimulate RNAP II release and directly interact with BRG1, the ATPase subunit of the chromatin remodelling complex SWI/SNF[70,71]. Upon heat shock, RNAP II is released from its paused state, leading to the synthesis of a full-length transcript. Rapid disruption of nucleosomes occurs across the entire *HSP70* gene, at a rate that is faster than RNAP II-mediated transcription[72]. The nucleosome displacement occurs simultaneously with HSF recruitment to the promoter in *D. melanogaster*. Downregulation of HSF abrogates the loss of nucleosomes, indicating that HSF provides a signal for chromatin rearrangement, which is required for *HSP70* nucleosome displacement. Within seconds of heat shock, the amount of HSF at the promoter increases drastically and HSF translocates from the nucleoplasm to several native loci, including HSP genes. Interestingly, the levels of HSF occupying the *HSP70* promoter reach saturation soon after just one minute[65,73].

HSF recruits the co-activating mediator complex to the heat shock loci, which acts as a bridge to transmit activating signals from transcription factors to the basal transcription machinery. The mediator complex is recruited by a direct interaction with HSF: the transactivation domain of *D. melanogaster* HSF binds to TRAP80 (also known as MED17), a subunit of the mediator complex[74]. HSF probably has other macromolecular contacts with the preinitiation complex as it binds to TATA-binding protein (TBP) and the general transcription factor TFIIB *in vitro*[75,76]. In contrast to the rapid recruitment and elongation of RNAP II on heat shock, activated HSF exchanges very slowly at the *HSP70* promoter. HSF stays stably bound to DNA *in vivo* and no turnover or disassembly of transcription activator is required for successive rounds of *HSP70* transcription[65,68].

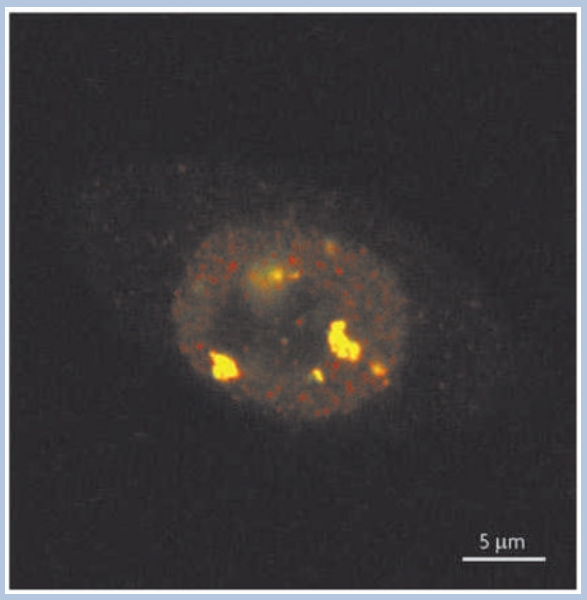
Functional interplay between HSFs

Although HSF1 is the principal regulator of the heat shock response, HSF2 also binds to the promoters of HSP genes. In light of our current knowledge, HSF2 strictly depends on HSF1 for its stress-related functions as it is recruited to HSP gene promoters only in the presence of HSF1 and this cooperation requires an intact HSF1 DBD[77]. Nevertheless, HSF2 modulates, both positively and negatively, the HSF1-mediated inducible expression of HSP genes, indicating that HSF2 can actively participate in the transcriptional regulation of the heat shock response. Coincident with the stress-induced transcription of HSP genes, HSF1 and HSF2 colocalize and accumulate rapidly on stress into nuclear stress bodies (NSBs; BOX 2), where they bind to a subclass of satellite III repeats, predominantly in the human chromosome 9q12 (REFS 78-80). Consequently, large and stable non-coding satellite III transcripts are synthesized in an HSF1-dependent manner in NSBs[81,82]. The function of

these transcripts and their relationship with other HSF1 targets, and the heat shock response in general, remain to be elucidated.

Box 2

Nuclear stress bodies



The cell nucleus is highly compartmentalized and dynamic. Many nuclear factors are diffusely distributed throughout the nucleoplasm, but they can also accumulate in distinct subnuclear compartments, such as nucleoli, speckles, Cajal bodies and promyelocytic leukaemia (PML) bodies[136]. Nuclear stress bodies (NSBs) are different from any other known nuclear bodies[137,138]. Although NSBs were initially thought to contain aggregates of denatured proteins and be markers of heat-shocked cells, their formation can be elicited by various stresses, such as heavy metals and proteasome inhibitors[137]. NSBs are large structures, 0.3–3 μm in diameter, and are usually located close to the nucleoli or nuclear envelope[137,138]. NSBs consist of two populations: small, brightly stained bodies and large, clustered and ring-like structures[137].

NSBs appear transiently and are the main site of heat shock factor 1 (HSF1) and HSF2 accumulation in stressed human cells[80]. HSF1 and HSF2 form a physically interacting complex and colocalize into small and barely detectable NSBs after only five minutes of heat shock, but the intensity and size of NSBs increase after hours of continuous heat shock. HSF1 and HSF2 colocalize in HeLa cells that have been exposed to heat shock for one hour at 42°C (see the figure; confocal microscopy image with HSF1–green fluorescent protein in green and endogenous HSF2 in red). NSBs form on specific chromosomal loci, mainly on q12 of human chromosome 9, where HSFs bind to a subclass of satellite III repeats[78,79,83]. Stress-inducible and HSF1-dependent transcription of satellite III repeats has been shown to produce non-coding RNA molecules, called satellite III transcripts[81,82]. The 9q12 locus consists of pericentromeric heterochromatin, and the satellite III repeats provide scaffolds for docking components, such as splicing factors and other RNA-processing proteins[139–143].

HSF2 also modulates the heat shock response through the formation of heterotrimers with HSF1 in the NSBs when bound to the satellite III repeats[83] (FIG. 4). Studies on the

functional significance of heterotrimerization indicate that HSF1 depletion prevents localization of HSF2 to NSBs and abolishes the stress-induced synthesis of satellite III transcripts. By contrast, increased expression of HSF2 leads to its own activation and the localization of both HSF1 and HSF2 to NSBs, where transcription is spontaneously induced in the absence of stress stimuli. These results suggest that HSF2 can incorporate HSF1 into a transcriptionally competent heterotrimer[83]. It is possible that the amounts of HSF2 available for heterotrimerization with HSF1 influence stress-inducible transcription, and that HSF1–HSF2 heterotrimers regulate transcription in a temporal manner. During the acute phase of heat shock, HSF1 is activated and HSF1–HSF2 heterotrimers are formed, whereas upon prolonged exposures to heat stress the levels of HSF2 are diminished, thereby limiting heterotrimerization[83]. Intriguingly, in specific developmental processes such as corticogenesis and spermatogenesis, the expression of HSF2 increases spatiotemporarily, leading to its spontaneous activation. Therefore, it has been proposed that HSF-mediated transactivation can be modulated by the levels of HSF2 to provide a switch that integrates the responses to stress and developmental stimuli[83] (FIG. 4). Functional relationships between different HSFs are emerging, and the synergy of DNA-binding activities among HSF family members offers an efficient way to control gene expression in a cell- and stimulus-specific manner to orchestrate the differential upstream signalling and target-gene networks.

A new member of the mammalian HSF family, mouse HSF3, was recently identified[10]. Avian HSF3 was shown to be activated at higher temperatures and with different kinetics than HSF1 (REF. 84), whereas in mice, heat shock induces the nuclear translocation of HSF3 and activation of stress-responsive genes other than HSP genes[10]. Future experiments will determine whether HSF3 is capable of interacting with other HSFs, potentially through heterocomplex formation. HSF4 has not been implicated in the heat shock response, but it competes with HSF1 for common target genes in mouse lens epithelial cells[85], which will be discussed below. It is important to elucidate whether the formation of homotrimers or hetero trimers between different family members is a common theme in HSF-mediated transcriptional regulation.

HSFs as developmental regulators

Evidence is accumulating that HSFs are highly versatile transcription factors that, in addition to protecting cells against proteotoxic stress, are vital for many physioogical functions, especially during development. The initial observations using deletion experiments of the *D. melanogaster Hsf* gene revealed defective oogenesis and larvae development[86]. These effects were not caused by obvious changes in HSP gene expression patterns, which is consistent with the subsequent studies showing that basal expression of HSP genes during mouse embryogenesis is not affected by the lack of HSF1 (REF. 13). These results are further supported by genome-wide gene expression studies revealing that numerous genes, not classified as HSP genes or molecular chaperones, are under HSF1-dependent control[87,88].

Although mice lacking HSF1 can survive to adulthood, they exhibit multiple defects, such as increased prenatal lethality, growth retardation and female infertility[13]. Fertilized oocytes do not develop past the zygotic stage when HSF1-deficient female mice are mated with wild-type male mice, indicating that HSF1 is a maternal factor that is essential for early post-fertilization development[89]. Recently, it was shown that HSF1 is abundantly expressed in maturing oocytes, where it regulates specifically *Hsp90α* transcription[90]. The HSF1-deficient oocytes are devoid of HSP90α and exhibit a blockage of meiotic maturation, including delayed G2–M transition or germinal vesicle breakdown and defective asymmetrical division[90]. Moreover, intra-ovarian HSF1-depleted oocytes contain

dysfunctional mitochondria and are sensitive to oxidative stress, leading to reduced survival[91]. The complex phenotype of *Hsf1*-knockout mice also demonstrates the involvement of HSF1 in placenta formation, placode development and the immune system[15,85,92,93], further strengthening the evidence for a protective function of HSF1 in development and survival.

Both HSF1 and HSF2 are key regulators in the developing brain and in maintaining proteostasis in the central nervous system. Disruption of *Hsf1* results in enlarged ventricles, accompanied by astrogliosis, neurodegeneration, progressive myelin loss and accumulation of ubiquitylated proteins in specific regions of the postnatal brain under non-stressed conditions[94,95]. The expression of HSP25 (also known as HSPB1) and α-crystallin B chain (CRYAB), which are known to protect cells against stress-induced protein damage and cell death, is dramatically decreased in brains lacking HSF1 (REF. 13). In contrast to HSF1, HSF2 is already at peak levels during early brain development in mice and is predominantly expressed in the proliferative neuronal progenitors of the ventricular zone and post-mitotic neurons of the cortical plate[96–99]. HSF2-deficient mice have enlarged ventricles and defects in cortical lamination owing to abnormal neuronal migration[97–99]. Incorrect positioning of superficial neurons during cortex formation in HSF2-deficient embryos is caused by decreased expression of the cyclin-dependent kinase 5 (CDK5) activator p35, which is a crucial regulator of the cortical migration signalling pathway[100,101]. The *p35* gene was identified as the first direct target of HSF2 in cortex development[99]. As correct cortical migration requires the coordination of multiple signalling molecules, it is likely that HSF2, either directly or indirectly, also regulates other components of the same pathway.

Cooperativity of HSFs in development

In adult mice, HSF2 is most abundantly expressed in certain cell types of testes, specifically pachytene spermatocytes and round spermatids[102]. The cell-specific expression of HSF2 in testes is regulated by a microRNA, miR-18, that directly binds to the 3′ untranslated region (UTR) of HSF2 (J.K. Björk, A. Sandqvist, A.N. Elsing, N. Kotaja and L.S., unpublished observations). Targeting of HSF2 in spermatogenesis reveals the first physiological role for miR-18, which belongs to the oncomir-1 cluster associated mainly with tumour progression[103]. In accordance with the expression pattern during the maturation of male germ cells, HSF2-null male mice display several abnormal features in spermatogenesis, ranging from smaller testis size and increased apoptosis at the pachytene stage to a reduced amount of sperm and abnormal sperm head shape[97,98,104]. A genome-wide search for HSF2 target promoters in mouse testis revealed the occupancy of HSF2 on the sex chromosomal multi-copy genes spermiogenesis specific transcript on the Y 2 (*Ssty2*), *Sycp3*-like Y-linked (*Sly*) and *Sycp3*-like X-linked (*Slx*), which are important for sperm quality[104]. Compared with the *Hsf2*-knockout phenotype, disruption of both *Hsf1* and *Hsf2* results in a more pronounced phenotype, including larger vacuolar structures, more widely spread apoptosis and a complete lack of mature spermatozoa and male sterility[105]. The hypo thesis that the activities of HSF1 and HSF2 are intertwined and essential for spermatogenesis is further supported by our results that HSF1 and HSF2 synergistically regulate the sex chromosomal multi-copy genes in post-meiotic round spermatids (M.Å., A. Vihervaara, E.S. Christians, E. Henriksson and L.S., unpublished observations). Given that the sex chromatin mostly remains silent after meiosis, HSF1 and HSF2 are currently the only known transcriptional regulators during post-meiotic repression. These results, together with the earlier findings that HSF2 can also form heterotrimers with HSF1 in testes[83], strongly suggest that HSF1 and HSF2 act in a heterocomplex and fine-tune transcription of their common target genes during the maturation of male germ cells.

HSF1 and HSF4 are required for the maintenance of sensory organs, especially when the organs are exposed to environmental stimuli for the first time after birth[85,88]. During the

early postnatal period, *Hsf1*-knockout mice display severe atrophy of the olfactory epithelium, increased accumulation of mucus and death of olfactory sensory neurons[88]. Although lens development in HSF4-deficient mouse embryos is normal, severe abnormalities, including inclusion-like structures in lens fibre cells, appear soon after birth and the mice develop cataracts[85,106,107]. Intriguingly, inherited severe cataracts occurring in Chinese and Danish families have been associated with a mutation in the DBD of HSF4 (REF. 108). In addition to the established target genes, *Hsp25*, *Hsp70* and *Hsp90*, several new targets for HSF1 and HSF4, such as crystallin γF (*Crygf*), fibroblast growth factor 7 (*Fgf7*) and leukaemia inhibitory factor (*Lif*) have been found to be crucial for sensory organs[85,88]. Furthermore, binding of either HSF1 or HSF4 to the *Fgf7* promoter shows opposite effects on gene expression, suggesting competitive functions between the two family members[85]. In addition to the proximal promoters, HSF1, HSF2 and HSF4 bind to other genomic regions (that is, introns and distal parts of protein-coding genes in mouse lens), and there is also evidence for either synergistic interplay or competition between distinct HSFs occupying the target-gene promoters[109]. It is possible that the different HSFs are able to compensate for each other to some extent. Thus, the identification of novel functions and target genes for HSFs has been a considerable step forward in understanding their regulatory mechanisms in development.

HSFs and lifespan

The lifespan of an organism is directly linked to the health of its tissues, which is a consequence of the stability of the proteome and functionality of its molecular machineries. During its lifetime, an organism constantly encounters environmental and physiological stress and requires an efficient surveillance of protein quality to prevent the accumulation of protein damage and the disruption of proteostasis. Proteotoxic insults contribute to cellular ageing, and numerous pathophysiological conditions, associated with impaired protein quality control, increase prominently with age[11]. From studies on the molecular basis of ageing, in which a wide range of different model systems and experimental strategies have been used, the insulin and insulin-like growth factor 1 receptor (IGF1R) signalling pathway, which involves the phosphoinositide 3-kinase (PI3K) and AKT kinases and the Forkhead box protein O (FOXO) transcription factors (such as DAF-16 in *Caenorhabditis elegans*), has emerged as a key process. The downregulation of HSF reduces the lifespan and accelerates the formation of protein aggregates in *C. elegans* carrying mutations in different components of the IGF1R-mediated pathway. Conversely, inhibition of IGF1R signalling results in HSF activation and promotes longevity by maintaining proteostasis[110,111]. These results have prompted many laboratories that use other model organisms to investigate the functional relationship between HSFs and the IGF1R signalling pathway.

The impact of HSFs on the lifespan of whole organisms is further emphasized by a recent study, in which proteome stability was examined during *C. elegans* ageing[112]. The age-dependent misfolding and downregulation of distinct metastable proteins, which display temperature-sensitive missense mutations, was examined in different tissues. Widespread failure in proteostasis occurred rapidly at an early stage of adulthood, coinciding with the severely impaired heat shock response and unfolded protein response[112]. The age-dependent collapse of proteostasis could be restored by overexpression of HSF and DAF-16, strengthening the evidence for the unique roles of these stress-responsive transcription factors to prevent global instability of the proteome.

Limited food intake or caloric restriction is another process that is associated with an enhancement of lifespan. In addition to promoting longevity, caloric restriction slows down the progression of age-related diseases such as cancer, cardiovascular diseases and metabolic disorders, stimulates metabolic and motor activities, and increases resistance to

environmental stress stimuli[113]. To this end, the dynamic regulation of HSF1 by the NAD^+-dependent protein deacetylase SIRT1, a mammalian orthologue of the yeast transcriptional regulator Sir2, which is activated by caloric restriction and stress, is of particular interest. Indeed, SIRT1 directly deacetylates HSF1 and keeps it in a state that is competent for DNA binding. During ageing, the DNA-binding activity of HSF1 and the amount of SIRT1 are reduced. Consequently, a decrease in SIRT1 levels was shown to inhibit HSF1 DNA-binding activity in a cell-based model of ageing and senescence[42]. Furthermore, an age-related decrease in the HSF1 DNA-binding activity is reversed in cells exposed to caloric restriction[114]. These results indicate that HSF1 and SIRT1 function together to protect cells from stress insults, thereby promoting survival and extending lifespan. Impaired proteostasis during ageing may at least partly reflect the compromised HSF1 activity due to lowered SIRT1 expression.

Impact of HSFs in disease

The heat shock response is thought to be initiated by the presence of misfolded and damaged proteins, and is thus a cell-autonomous response. When exposed to heat, cells in culture, unicellular organisms, and cells in a multicellular organism can all trigger a heat shock response autonomously[115–117]. However, it has been proposed that multicellular organisms sense stress differently to isolated cells. For example, the stress response is not properly induced even if damaged proteins are accumulated in neurodegenerative diseases like Huntington's disease and Parkinson's disease, suggesting that there is an additional control of the heat shock response at the organismal level[118]. Uncoordinated activation of the heat shock response in cells in a multicellular organism could cause severe disturbances of interactions between cells and tissues. In *C. elegans*, a pair of thermosensory neurons called AFDs, which sense and respond to temperature, regulate the heat shock response in somatic tissues by controlling HSF activity[119,120]. Moreover, the heat shock response in *C. elegans* is influenced by the metabolic state of the organism and is reduced under conditions that are unfavourable for growth and reproduction[121]. Neuronal control may therefore allow organisms to coordinate the stress response of individual cells with the varying metabolic requirements in different tissues and developmental stages. These observations are probably relevant to diseases of protein misfolding that are highly tissue-specific despite the often ubiquitous expression of damaged proteins and the heat shock response.

Elevated levels of HSF1 have been detected in several types of human cancer, such as breast cancer and prostate cancer[122,123]. Mice deficient in HSF1 exhibit a lower incidence of tumours and increased survival than their wild-type counterparts in a classical chemical skin carcinogenesis model and in a genetic model expressing an oncogenic mutation of p53. Similar results have been obtained in human cancer cells lines, in which HSF1 was depleted using an RNA interference strategy[124]. HSF1 expression is likely to be crucial for non-oncogene addiction and the stress phenotype of cancer cells, which are attributes given to many cancer cells owing to their high intrinsic level of proteotoxic and oxidative stress, frequent spontaneous DNA damage and aneuploidy[125]. Each of these features may disrupt proteostasis, raising the need for efficient chaperone and proteasome activities. Accordingly, HSF1 would be essential for the survival of cancer cells that experience constant stress and develop non-oncogene addiction.

HSFs as therapeutic targets

Given the unique role of HSF1 in stress biology and proteostasis, enhanced activity of this principal regulator during development and early adulthood is important for the stability of the proteome and the health of the cell. However, HSF1 is a potent modifier of tumorigenesis and, therefore, a potential target for cancer therapeutics[125]. In addition to modulating the expression of HSF1, the various PTMs of HSF1 that regulate its activity

should be considered from a clinical perspective. As many human, age-related pathologies are associated with stress and misfolded proteins, several HSF-based therapeutic strategies have been proposed[126]. In many academic and industrial laboratories, small molecule regulators of HSF1 are actively being searched for (see Supplementary information S1 (table)). For example, celastrol, which has antioxidant properties and is a natural compound derived from the Celastreace family of plants, activates HSF1 and induces HSP expression with similar kinetics to heat shock, and could therefore be a potential candidate molecule for treating neurodegenerative diseases[127,128]. In a yeast-based screen, a small-molecule activator of human HSF1 was found and named HSF1A[129]. HSF1A, which is structurally distinct from the other known activators, activates HSF1 and enhances chaperone expression, thereby counteracting protein misfolding and cell death in polyQ-expressing neuronal precursor cells[129]. Triptolide, also from the Celastreace family of plants, is a potent inhibitor of the transactivating capacity of HSF1 and has been shown to have beneficial effects in treatments of pancreatic cancer xenografts[130,131]. These examples of small-molecule regulators of HSF1 are promising candidates for drug discovery and development. However, the existence of multiple mammalian HSFs and their functional interplay should also be taken into consideration when planning future HSF-targeted therapies.

Concluding remarks and future perspectives

HSFs were originally identified as specific heat shock-inducible transcriptional regulators of HSP genes, but now there is unambiguous evidence for a wide variety of HSF target genes that extends beyond the molecular chaperones. The known functions governed by HSFs span from the heat shock response to development, metabolism, lifespan and disease, thereby integrating pathways that were earlier strictly divided into either cellular stress responses or normal physiology.

Although the extensive efforts from many laboratories focusing on HSF biology have provided a richness of understanding of the complex regulatory mechanisms of the HSF family of transcription factors, several key questions remain. For example, what are the initial molecular events (that is, what is the ‘thermometer’) leading to the multistep activation of HSFs? The chromatin-based interaction between HSFs and the basic transcription machinery needs further investigation before the exact interaction partners at the chromatin level can be established. The activation and attenuation mechanisms of HSFs require additional mechanistic insights, and the roles of the multiple signal transduction pathways involved in post-translational regulation of HSFs are only now being discovered and are clearly more complex than anticipated. Although still lacking sufficient evidence, the PTMs probably serve as rheostats to allow distinct forms of HSF-mediated regulation in different tissues during development. Further emphasis should therefore be placed on understanding the PTMs of HSFs during development, ageing and different protein folding diseases. Likewise, the subcellular distribution of HSF molecules, including the mechanism by which HSFs shuttle between the cytoplasm and the nucleus, remains enigmatic, as do the movements of HSF molecules in different nuclear compartments such as NSBs.

Most studies on the impact of HSFs in lifespan and disease have been conducted with model organisms such as *D. melanogaster* and *C. elegans*, which express a single HSF. The existence of multiple members of the HSF family in mammals warrants further investigation of their specific and overlapping functions, including their extended repertoire of target genes. The existence of multiple HSFs in higher eukaryotes with different expression patterns suggests that they may have functions that are triggered by distinct stimuli, leading to activation of specific target genes. The impact of the HSF family in the adaptation to diverse biological environments is still poorly understood, and future studies are likely to broaden the prevailing view of HSFs being solely stress-inducible factors. To this end, the

crosstalk between distinct HSFs that has only recently been uncovered raises obvious questions about the stoichiometry between the components in different complexes residing in different cellular compartments, and the mechanisms by which the factors interact with each other. Interaction between distinct HSF family members could generate new opportunities in designing therapeutics for protein-folding diseases, metabolic disorders and cancer.

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Glossary

Polytene chromosome	A chromosome that undergoes multiple rounds of DNA replication, without cell division, and produces many sister chromatids that remain synapsed together;for example in larval salivary glands of *D. melanogaster*
Proteostasis	Also called protein homeostasis, this refers to the control of the concentration, three-dimensional structure, binding interactions and cellular location of individual proteins making up the proteome
S1 mapping	A method for mapping precursor or mature mRNAs that correspond to particular DNA sequences using the S1 nuclease enzyme
Coiled coil	A structural motif in proteins, in which α-helices are coiled together like the strands of a rope, most commonly as dimers and trimers
Leu zipper	A common three-dimensional structural motif in regulatory proteins that functions as an oligomerization domain and generates adhesion forces in parallel α-helices
ΨKxExxSP	Many SUMO substrates contain this extended consensus sequence (called the PDSM), in which Ψ denotes a branched hydrophobic amino acid, the Lys is the SUMO acceptor and x is any amino acid
Sirtuin	A class of proteins that posses either histone deacetylase or monoribosyltransferase activity. SIRT1 is activated by resveratrol, which is a phytoalexin produced naturally by several plants when under attack by pathogens, and has been suggested to trigger mechanisms that counteract ageing-related effects in animals
Paused RNA polymerase II	An RNA polymerase II molecule that is engaged in transcription but has arrested after synthesizing ~25 nucleotides; for example on the *HSP70* promoter under non-heat-shock conditions
Mediator complex	A multiprotein complex that functions as a transcriptional co-activator and binds to the C-terminal domain of the RNA polymerase II holoenzyme, acting as a bridge between this enzyme and transcription factors
Preinitiation complex	A large protein complex that is necessary for the transcription of protein-coding genes in eukaryotes and typically consists of six

	general transcription factors; TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH
Satellite III repeat	A highly repetitive DNA element that is so called because repetitions of a short DNA sequence tend to produce a different frequency of the nucleotides adenine, cytosine, guanine and thymine compared with bulk DNA
Germinal vesicle breakdown	The process whereby a greatly enlarged nucleus of an egg in prophase of the first meiotic division breaks down, permitting entry into metaphase I
Cortical plate	The middle layer of the cerebral cortex. The cerebral cortex is formed during development, and neurons migrate from the ventricular zone to form layers. The cortical plate will form the deep layers of the mature cortex
Ventricle	A brain structure that contains cerebrospinal fluid. There are four cerebral ventricles: the paired lateral ventricles that are large and C shaped, and the third and fourth ventricles in the midline
Cortical lamination	The characteristic distribution of neuronal cell types and their connections in the six main layers of the cerebral cortex
Neuronal migration	The process whereby neurons migrate from their place of origin or birth to their final position in the brain by, for example, radial migration or tangential migration
Pachytene spermatocyte	A male gametocyte that derives from a spermatogonium, resides in the seminiferous tubules of the testis and is in the developmental stage of spermatogenesis, before which meiosis occurs
Round spermatid	A haploid male gamete derived from spermatocyte division. As a result of meiosis, each round spermatid contains only half of the genetic material present in the original spermatocyte
Sex chromosomal multi-copy gene	A large amplified region that comprises palindromic or tandem segmental duplications, contains multi-copy gene families and resides in the X and Y chromosomes
Sex chromatin	The complex combination of DNA and proteins that makes up chromatin of the X and Y chromosomes during spermatogenesis
Cataract	Clouding that develops in the crystalline lens of the eye or in its envelope, varying in degree from slight to complete opacity and obstructing the passage of light
Unfolded protein response	A cellular stress response that is activated by an accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum
Non-oncogene addiction	The increased dependence of cancer cells on the normal cellular functions of certain genes, which are not classical cancer genes or oncogenes but, if targeted, they could be equally effective at treating cancer
Aneuploidy	An abnormal number of chromosomes generated during cell division when the chromosomes do not separate properly between the two daughter cells. This is a characteristic of cancer cells

PolyQ	A protein sequence that consists of multiple (typically tens to hundreds) Glu repeats. Several inheritable neurodegenerative disorders are polyQ diseases, characterized by a mutation that extends a CAG (which encodes Glu) repeat in a specific gene (for example, huntingtin (*HTT*) in Huntington's disease) beyond a certain length
Rheostat	An adjustment device (a term borrowed from electronics)

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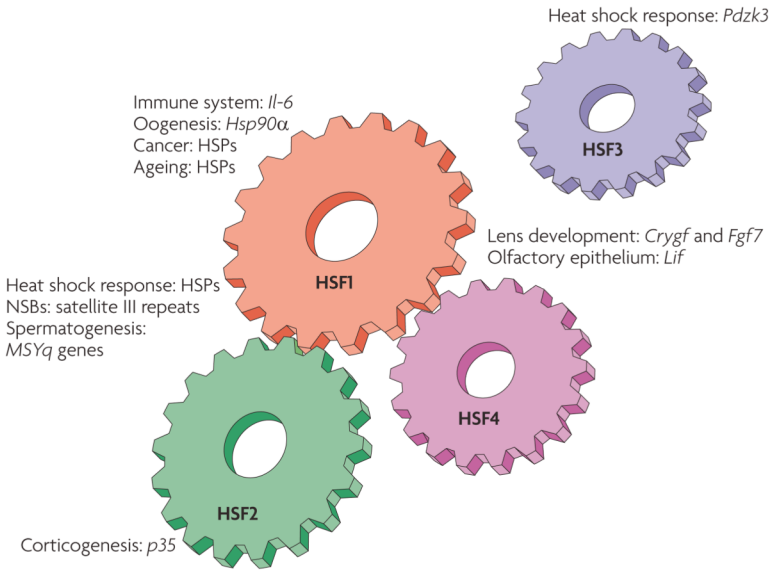

Figure 1. The mammalian HSF machinery

An overview of the mammalian heat shock factor (HSF) family members and their biological functions. HSFs contribute to multiple normal physiological processes and pathologies through direct regulation of their target genes. The HSF target genes that have been identified *in vivo* are shown. HSF1 was originally recognized as the principal stress-responsive regulator of the heat shock response, but now HSF2 is known to modulate HSF1-mediated expression of heat shock protein (HSP) genes through heterocomplex formation. On heat shock, HSF1 and HSF2 accumulate into nuclear stress bodies (NSBs), where they bind to satellite III repeats. HSF1 is also a regulator of immune responses and cancer. So far, the regulation of HSP genes in ageing has most intensively been examined in *Caenorhabditis elegans*. Both HSF1 and HSF2 have been ascribed regulatory functions in several developmental processes, such as oogenesis, spermatogenesis and corticogenesis. HSF4 is involved in the development of different sensory organs in cooperation with HSF1, but has no role in the heat shock response. Murine HSF3 is the most recently identified mammalian HSF, which participates in the heat shock response by binding to the PDZ domain-containing 3 (*Pdzk3*) promoter[10]. Currently, HSF3 is not known to crosstalk with any member of the HSF family, and is therefore placed separately from the other HSFs. *Crygf*, crystallin γF; *Fgf7*, fibroblast growth factor 7; *Il-6*, interleukin-6; *MSYq*, male-specific long arm of the mouse Y chromosome.

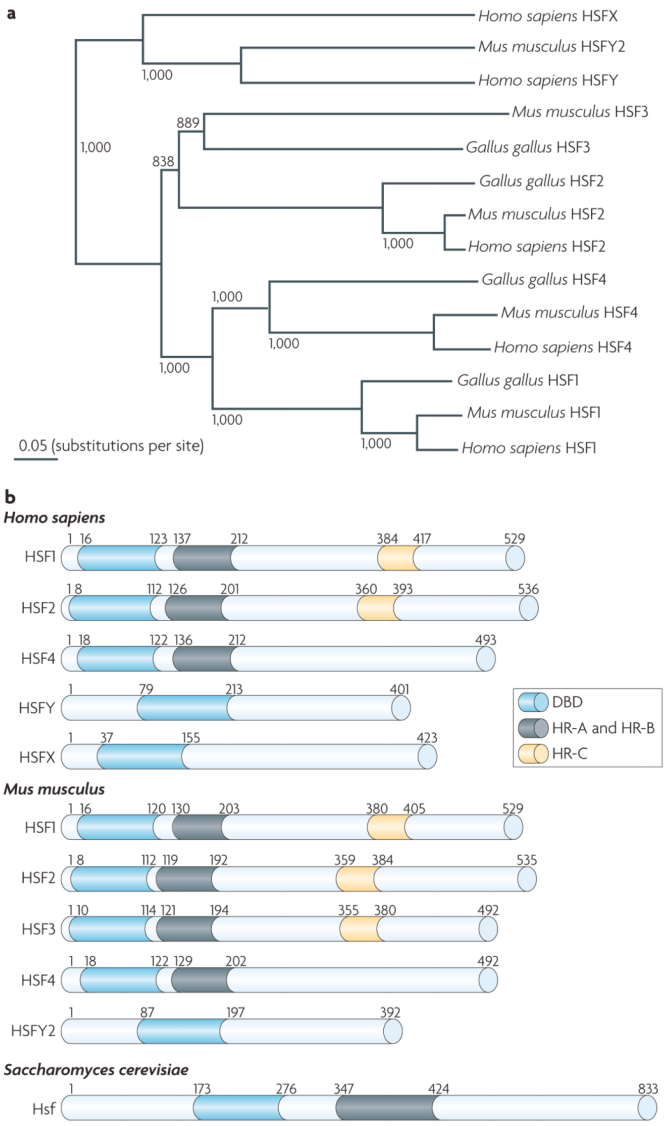

Figure 2. Members of the mammalian HSF family

a | A phylogenetic tree showing the species-specific relationship of heat shock factors (HSFs) among higher eukaryotes. Two recently found, but still poorly characterized, family members are: HSFY, which is located on the human Y chromosome and on the murine chromosome 2 (HSFY2), and HSFX, which has only been found on the human X chromosome[144–146]. HSFY and HSFX exist in two identical copies on their respective chromosome. The phylogenetic tree was generated in CLUSTAL W[147] and gaps were excluded from all phylogenetic analyses. The numbers represent bootstrap values (1000 bootstrap replicates were carried out). **b** | A schematic of the functional domains of the human and murine HSF family members. The conserved domains of distinct HSFs are indicated: the DNA-binding domain (DBD), the oligomerization domain (heptad repeat A (HR-A) and HR-B) and the carboxy-terminal HR-C. All HSFs contain the characteristic helix-loop-helix DBD. HSF1–HSF4 contain Leu zipper-like HR domains, which are required for homotrimerization or heterotrimerization. Yeast Hsf is included as a comparison. Image in part **a** is modified, with permission, from REF. 10 © (2010) The American Society for Cell Biology.

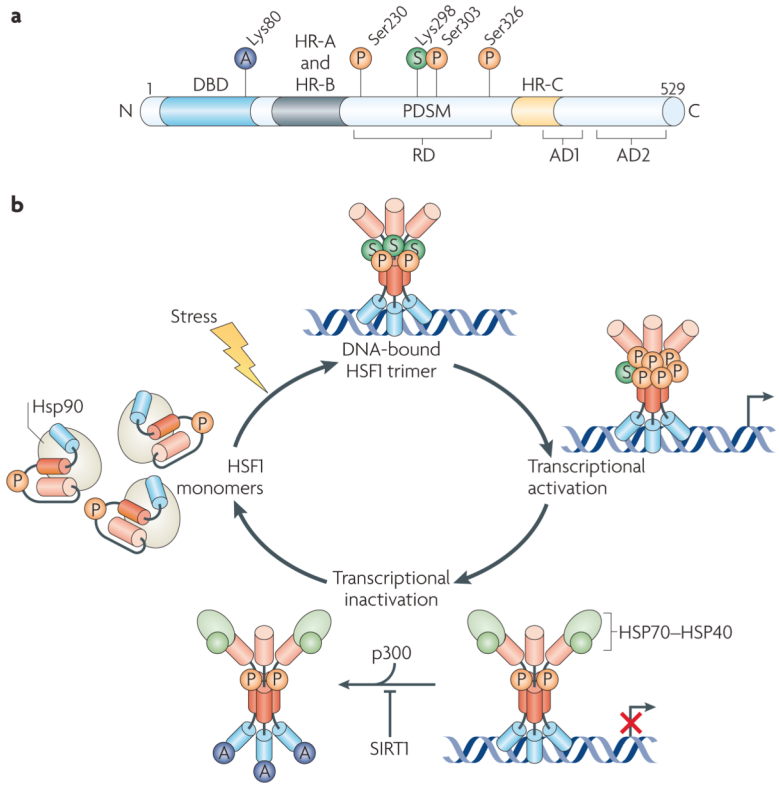

Figure 3. HSF1 undergoes multiple PTMs on activation

a | An overview of heat shock factor 1 (HSF1)-related post-translational modifications (PTMs). Some of the identified sites for acetylation (A), phosphorylation (P) and sumoylation (S) are indicated, as well as the phosphorylation-dependent sumoylation motif (PDSM). The DNA-binding domain (DBD) and the heptad repeats (HR-A and HR-B, and HR-C) are indicated as in FIG. 2, as well as the regulatory domain (RD) and activation domains (AD1 and AD2). **b** | The HSF1 activation and attenuation cycle, involving trimerization, multiple PTMs and feedback from heat shock proteins (HSPs). In the resting state, HSF1 is a monomer in both the cytoplasm and nucleus. Monomeric HSF1 is already a phosphoprotein under non-stress conditions and it interacts with HSP90. On stress, HSF1 dissociates from the HSP90 complex, allowing HSF1 to trimerize and bind to the heat shock elements (HSEs) in HSP genes. Several PTMs, such as phosphorylation and sumoylation, are involved in regulating the transactivation capacity of HSF1. HSF1 acquires transcriptional activity, which is abrogated during the attenuation phase. Attenuation involves two regulatory steps: negative feedback from HSPs, which represses the transactivation of DNA-bound HSF1, and inhibition of DNA binding by the acetylation of Lys80 in the DBD of HSF1. The sirtuin SIRT1 regulates the attenuation phase of the heat shock response by preventing HSF1 acetylation[42].

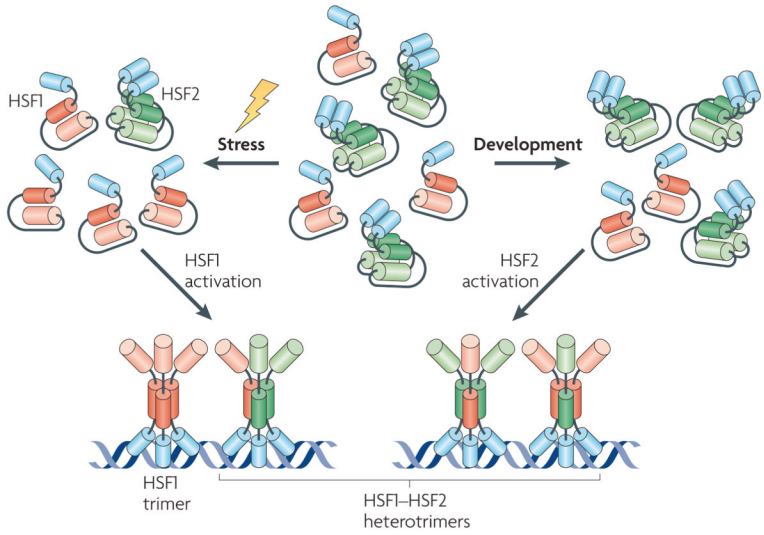

Figure 4. Interactions between different HSFs provide distinct functional modes in transcriptional regulation

On stress, heat shock factor 1 (HSF1) is activated and HSF1–HSF2 heterotrimers are formed. Heat shock stress diminishes the levels of HSF2 and restricts heterotrimerization by limiting the availability of HSF2. Biochemical characterization of HSF2 has revealed that, unlike HSF1, which undergoes a monomer-to-trimer transition, HSF2 is mainly converted from a dimer to a trimer on activation[148]. In certain developmental processes, such as corticogenesis and spermatogenesis, HSF2 levels are elevated in specific cell types and tissues, leading to activation of HSF2. Increased HSF2 expression then induces the formation of heterotrimers with HSF1. It has therefore been suggested that HSF1–HSF2 heterotrimerization provides a switch that integrates the transcriptional activation in response to specific stimuli[83].