

Heat Shock Factors at a Crossroad between Stress and Development

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ABSTRACT: Organisms must be able to sense and respond rapidly to changes in their environment in order to maintain homeostasis and survive. Induction of heat shock proteins (Hsps) is a common cellular defence mechanism for promoting survival in response to various stress stimuli. Heat shock factors (HSFs) are transcriptional regulators of Hsps, which function as molecular chaperones in protecting cells against proteotoxic damage. Mammals have three different HSFs which have been considered functionally distinct; HSF1 is essential for the heat shock response and is also required for developmental processes, whereas HSF2 and HSF4 are important for differentiation and development. Specifically, HSF2 is involved in corticogenesis and spermatogenesis, and HSF4 is needed for maintenance of sensory organs, such as the lens and the olfactory epithelium. Recent evidence, however, suggests a functional interplay between HSF1 and HSF2 in the regulation of *Hsp* expression under stress conditions. In lens formation, HSF1 and HSF4 have been shown to have opposite effects on gene expression. In this chapter, we present the different roles of the mammalian HSFs as regulators of cellular stress and developmental processes. We highlight the interaction between different HSFs and discuss the discoveries of novel target genes in addition to the classical *Hsps*.

KEYWORDS: Heat shock factor; heat shock response; corticogenesis; spermatogenesis; transcription

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THE MAMMALIAN HEAT SHOCK FACTOR FAMILY

The eukaryotic heat shock response (HSR) is mediated by a positive control element, the heat shock element (HSE), which is present in multiple copies upstream of the *Hsp* genes. The first evidence for a factor that could interact with the HSE originated from studies of protein-DNA interactions in *Drosophila* cell nuclei.¹ An activator protein, named heat shock factor (HSF), was identified to specifically bind to the HSE and regulate the *Hsp* expression upon stress stimulation. Since then efforts from a large number of investigators have shown that the HSR is conserved in all organisms from yeast to plants and animals. In yeast, fruit fly and nematode, only a single HSF exists, whereas in vertebrates and plants, the HSF family consists of several members.^{2,3} HSF1 and HSF2 exist in all vertebrates, while HSF3 is specific for avian species and HSF4 for mammals.⁴ HSF1 was originally identified as the transcriptional regulator of the HSR and has been most extensively investigated in mammals. HSF1 is activated in response to elevated temperatures, exposure to oxidants, heavy metals, and bacterial or viral infections, and genetic studies indicate that no other HSF is able to compensate for HSF1 in the HSR.⁵⁻⁷ HSF2 is known to be involved in development and differentiation-related processes such as spermatogenesis and corticogenesis in mice and hemin-mediated differentiation of human K562 erythroleukemia cells.⁸⁻¹⁴ No stress-related functions have been shown for HSF4, but its importance in lens formation and maintenance of olfactory epithelium has been well documented.¹⁵⁻¹⁷

HSF activation is a multistep process, including trimerization, localization to the nucleus, and binding to DNA. Several inducible post-translational modifications (PTMs), such as phosphorylation and sumoylation, are involved in regulation of the transactivation capacity of HSF1.¹⁸⁻²¹ Upon activation HSF1 undergoes a transition from monomer to trimer,^{22,23} whereas HSF2 undergoes a transition from dimer to trimer.⁹ Similar to most transcriptional regulators, HSFs are composed of different functional domains, of which the DNA-binding domain (DBD) is best preserved (Fig. 1.).^{21,23} HSFs bind to DNA where each DBD recognizes the HSE in the major groove of the double helix.²³ HSEs are highly conserved consisting of multiple inverted repeats of the pentameric sequence nGAAn.²⁴ The promoters of HSF target genes can also have more than one HSE, thereby allowing simultaneous binding of multiple HSFs. HSF binding to an HSE occurs in a cooperative manner, where binding of one HSF trimer facilitates the binding of the next.²⁵ Trinklein and colleagues confirmed the finding of Xiao and Lis, identifying guanines to be the most conserved nucleotides within the HSEs.^{26,27} In addition to typical HSEs, binding of HSF1 to a discontinuous type of HSE was recently observed *in vitro*.²⁸ These results suggest that both the nucleotides and the spacing of the repeated units are critical determinants for recognition by HSFs and transcriptional activation.

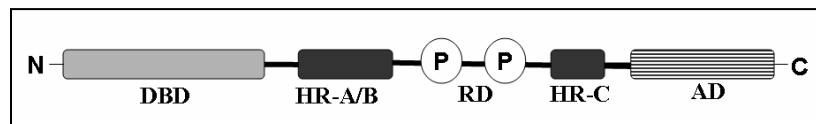


FIGURE 1. The functional domains of HSF1. DBD: DNA-binding domain, HR-A/B and HR-C: Hydrophobic heptad repeats, RD: Regulatory domain, AD: Activation domain, P: Post-translational modifications (PTMs). Note that PTMs occur also within other functional domains than RD.

HSFs AS CELLULAR STRESS REGULATORS

HSF1 is the *bona fide* stress-responsive prototype in mammals. *Hsf1* knockout mouse models have demonstrated that HSF1 is required as a transcriptional activator of *Hsp* genes during the HSR. Moreover, HSF1 is critical for maintaining cellular integrity during stress, since cells from *Hsf1*^{-/-} mice lack the ability to develop thermotolerance.^{5-7,29} In contrast, the role of HSF2 in the HSR was not revealed until very recently.¹⁴ *Hsf2*-null mice are viable and have no gross defects in the HSR, but several reports have proposed that HSF2 could contribute in the transcriptional regulation through interplay with HSF1.^{10-12,14,30-32}

Functional interaction between HSF1 and HSF2

Using chromatin immunoprecipitation (ChIP), binding of both HSF1 and HSF2 was detected on *Hsp* promoters upon heat shock and hemin treatment (TABLE 1).^{14,27} Further studies using both knockdown and knockout strategies elucidated that during stress, HSF2 is recruited to the *Hsp70* promoter only in the presence of HSF1 and that this cooperation requires an intact HSF1 DBD.¹⁴ Importantly, gene expression analyses showed that HSF2 is able to modulate the HSF1-mediated inducible expression of *Hsps* and reintroduction of HSF2 into *Hsf2*^{-/-} fibroblasts potentiated the expression of major *Hsp* genes. In addition, individual targets were differently regulated, depending on stimuli. These findings indicate that HSF2, in contrast to the previous model, actively participates in the transcriptional regulation of the HSR.¹⁴ HSF2 has also been reported to regulate the chromatin structure of the *Hsp70* promoter during mitosis,³³ and it will be interesting to find out whether HSF1, possibly through interplay with HSF2, would participate in bookmarking. HSF1 and HSF2 were also found to interact during proteasome inhibition, and both factors bound to the *clusterin* (*Clu*) promoter in such stress conditions.³² Since the HSE located on the *Clu* promoter contains only three pentamers, HSF1 and HSF2 binding as a heterocomplex is an intriguing possibility.

TABLE 1. Mammalian HSF target genes identified *in vivo*

DNA-binding factor	Target gene promoter	Reference
HSF1 and HSF2	<i>Hsps</i>	27
HSF1 and HSF2	<i>Hsp70.1</i> and <i>Hsp25.1</i>	14
HSF1 and HSF2	<i>Clu</i>	32
HSF1	<i>Il-6</i>	34
HSF4	<i>Crygf</i>	16
HSF1 and HSF4	<i>Fgf7</i>	16
HSF1 and HSF4	<i>Lif</i>	17
HSF2	<i>p35</i>	13

Apart from target gene promoters, HSF1 concentrates rapidly upon stress into nuclear stress bodies (nSBs). nSBs form on specific chromosomal loci, mainly q12 of human chromosome 9, where HSF1 binds to a subclass of satellite III repeats.^{35,36} Stress-inducible HSF1-dependent transcription of the satellite III repeats, originating from the 9q12 locus, has been shown to produce non-coding RNA molecules, whose functions remain to be established.³⁷ Intriguingly, HSF2 was also found to localize in nSBs in HeLa cells exposed to heat stress.³⁸ Upon heat shock, HSF1 and HSF2 co-localize in the nSBs,³⁹ and an interaction between HSF1 and HSF2 during both control and heat shock treatment has been detected,^{31,39} suggesting a possible functional interplay between the two transcription factors. Further studies are required to determine whether HSF2 functions as a modulator of HSF1-mediated transactivation of other targets than *Hsps*, including satellite III repeats, and whether competition between HSFs is a common phenomenon in the regulation of their target gene expression.

HSF1 in the immune response

Hsf1 knockout mice display a significantly impaired T cell-dependent B cell response.³⁴ Transcriptional profiling of *Hsf1*^{-/-} fibroblasts has revealed that HSF1, in addition to *Hsp* genes, regulates immunologically important genes. In mouse spleen cells, HSF1 was found to directly bind to the *Il-6* gene (TABLE 1.),³⁴ coding for a pro-inflammatory cytokine secreted by T cells to stimulate an immune response and is required for B cell differentiation.⁴⁰ In response to immunization with sheep red blood cells, the *Hsf1*-deficient mice showed 50% lower production of immunoglobulins, especially IgG2a. These results unravelled a novel molecular link between HSF1 and a gene related to immune response and inflammation.³⁴

The nematode *C. elegans* has evolved an immune system, which is excellent for studying the effect of elevated temperatures on immunity. Upon heat shock the worms become more resistant to bacterial pathogens. The enhanced resistance requires HSF1-mediated activation of Hsp90 and small Hsps, effectors of the immune protection.⁴¹ In addition, the HSF1 defence

pathway interacts with the insulin/IGF-1 signaling pathway, including FOXO transcription factor DAF-16 and its upstream receptor DAF-2 that are known to affect aging and immunity in *C. elegans*.⁴¹⁻⁴³ These findings indicate that HSF1 has multiple ways of regulating the immune system.

HSFs AS DEVELOPMENTAL REGULATORS

A developmental role for HSFs was introduced when the *Drosophila* HSF was found to be required for early larval development and oogenesis.⁴⁴ Surprisingly, these developmental effects were not mediated by *Hsp* gene expression, which is consistent with the subsequent studies showing that basal *Hsp* expression during mouse embryonic development is not affected by the lack of HSF1.⁶ Gene inactivation studies in mice have revealed functions beyond the HSR and demonstrated roles in embryonic development, reproduction, cortical lamination, lens development, and maintenance of olfactory epithelium.^{10,11,13,16,17,30,45} A major challenge is to establish the genes that are directly controlled by HSFs, and most importantly, the processes where these gene products play a key role.

HSF1 – a maternal factor

Mice lacking HSF1 can survive to adulthood but they exhibit multiple defects, including placental insufficiency, prenatal lethality, growth retardation, and female infertility.⁶ In developing *Hsf1*^{-/-} embryos, no extensive defects were evident and no changes were detected in the expression of *Hsp70*, which is the earliest sign of zygotic genome activation. In contrast, an abnormal architecture of the placenta was observed at E11.5, suggesting that the prenatal lethality was due to failure in the extra-embryonic tissue. No fertilized oocytes developed past the zygotic stage when *Hsf1*^{-/-} females were mated with wildtype males. These results demonstrate that HSF1 is a maternal factor, essential for early post-fertilization development.⁴⁶ Disturbed control by maternal HSF1 during oogenesis or in the initiation phase of embryogenesis could therefore be associated with infertility in mammals.

HSF1 and HSF4 interplay in sensory organs

Little was known about the physiological function of HSF4 before a genetic study by Bu and coworkers showed that inherited cataract in certain Chinese and Danish families was associated with a mutation in the DBD of HSF4.⁴⁷ The phenotype of *Hsf4*-null mice supports an important role for HSF4 in lens formation; although *Hsf4* knockout mice displayed normal lens development during embryogenesis, abnormalities in the lens appeared soon

after birth and the mice developed cataract by six weeks of age.^{16,45} *Hsf4*^{-/-} lens fiber cells were abnormal containing inclusion-like structures, probably due to a reduction in the expression of γ -crystallin gene family members. HSF4 was found to directly bind and regulate the γ F-crystallin (*Crygf*) gene.¹⁶ Binding of HSF1 and HSF4 to the *Fgf7* promoter showed opposite effects on gene expression, i.e. repression by HSF4 and activation by HSF1, providing evidence for a competition between these two HSFs during mouse lens development (TABLE 1).¹⁶ This finding is the first example of an interplay between two different mammalian HSFs in development.

During early post-natal period the *Hsf1*^{-/-} mice display severe atrophy of the olfactory epithelium, increased cell death of olfactory sensory neurons and increased expression of the *Lif* gene.¹⁷ In contrast, this phenotype is alleviated to some extent in the *Hsf4*^{-/-} olfactory epithelium. A similar interplay between HSF1 and HSF4 as detected during lens formation, also occurs on the *Lif* promoter in the olfactory epithelium (TABLE 1).^{16,17} HSF1 and HSF4 are required for the maintenance of different sensory organs, the lens and the olfactory epithelium, specifically when these organs are exposed to environmental stimuli for the first time after birth.^{16,17} The increased sensitivity of these organs may be partly due to the altered expression of *Crygf*, *Fgf7* and *Lif*. In addition, decreased levels of *Hsp25*, *Hsp70* and *Hsp90* were observed in *Hsf1*^{-/-} olfactory epithelium, and *Hsf4*-deficient lens fiber cells had compromised expression of *Hsp25*. The results suggest that the preservation of the protein homeostasis by Hsps could be an important determinant in sensory organ maintenance.^{16,17} Although these gene inactivation studies mainly focused on the cooperative and competitive roles of HSF1 and HSF4, it is also possible that HSF2 might have a function in the neuronal part of retinal formation, due to similar expression patterns.⁴⁸

HSFs in brain development

During rodent brain development, HSF2 is highly expressed in the neuroepithelium, with nuclear localization in the developing neural tube, and HSF2 DNA-binding activity can be detected in cortex, striatum, olfactory bulbs and mesencephalon before birth (Y. Chang's unpublished results).^{10,11,13,49-51} In the mouse, HSF2 is expressed in the proliferative neuronal progenitors of the ventricular zone. In addition, HSF2 expression is detected in the cortical plate, when the most superficial layers of cortex are being established.^{10,50} HSF2 expression and activity profiles implicate a major role for HSF2 as a transcriptional regulator in development of fore- and midbrain, and possibly also in cerebellum.

Hsf2 inactivation studies have been performed by three different laboratories.^{10,11,30} In all three cases, *Hsf2*-null mice did not display any overt morphological abnormalities. While one laboratory did not observe any brain phenotype in adult mice,³⁰ embryonic brain defects were reported by the two

others groups.^{10,11} Adult brains displayed enlarged ventricles and reduction of hippocampus and striatum, as well as in the width of the cortex. In addition, prominent abnormalities in the central nervous system (CNS), with collapse of the ventricular systems and hemorrhages in cerebral regions at early stages, were detected.^{10,11} HSF2 was also found to be involved in later brain development, in the migration phase of newborn cortical neurons.¹³ When migrating, cortical neurons receive migration inputs, such as Reelin secreted from Cajal-Retzius cells, and the neurons benefit from architectural guides provided by radial glia cell fibers, which extend all the way from the ventricular zone to the marginal zone.⁵² In the absence of HSF2, a reduced number of radial glia and Cajal-Retzius cells, together with disturbances in the Reelin signaling cascade, were observed.¹³ Moreover, the expression of p35, which is an activator of cyclin-dependent kinase 5 (Cdk5) and essential for radial migration,⁵² was found to be dependent on the amount of HSF2.¹³ As demonstrated *in vivo* by ChIP experiments, HSF2 directly bound to the promoter of p35, which was thereby identified as the first HSF2 target gene in development (TABLE 1.).¹³ In the light of present knowledge, HSF2 could function as a fine tuner of gene expression, required for correct neuronal positioning in superficial layers in the developing cortex. The role of HSF2 in cortical development is unlikely to be restricted only to the late phase of migration, as HSF2 also is expressed at high levels in the cells of the neuroepithelium and neuronal progenitors in the ventricular zone (D. Trouillet's unpublished results).^{10,11} It is plausible that HSF2 participates in the regulation of neuronal proliferation, which is well in line with defects observed in the early CNS of *Hsf2*-null mice.¹¹

Although no substantial data on the role of HSF1 in brain development is currently available, HSF1 has been implicated in maintenance of the post-natal brain under non-stressed conditions.⁵³ In accordance with *Hsf2*-null mice, *Hsf1* disruption resulted in enlarged ventricles. Moreover, astrogliosis and neurodegeneration occurred in specific areas.⁵³ Interestingly, the expression levels of *Hsp27* and *α B crystallin*, which protect cells against stress and apoptosis, were decreased in *Hsf1* knockout brain regions. Since *Hsf1*^{-/-} embryonic brains are still normal at E18.5, the abnormalities probably originate from a later stage in the perinatal and post-natal development.⁶

HSFs in spermatogenesis

HSFs have been found to be involved in the regulation of gametogenesis in both genders.^{10-12,46,54,55} In males, experimental evidence reveals a critical function for both HSF1 and HSF2 in germ cell production. A constitutively active form of HSF1 caused disruption of spermatogenesis and death of pachytene spermatocytes.⁵⁴ Although *Hsf1*^{-/-} mice are fertile and exhibit normal spermatogenesis, decreased heat-induced elimination of the pachytene spermatocytes was observed, which is an opposite effect to that detected in HSF1-overexpressing mice.⁵⁵ In general, mutations affecting

spermatogenesis result in apoptosis at the pachytene stage,⁵⁶ and HSF1 is activated at this specific stage, which could be a marker for accumulation of damaged proteins and a signal to induce cell death.⁵⁵

Hsf2 deficiency resulted in reduced size of testis, increased apoptosis and decreased sperm count.^{10,11} Severe disruption and vacuolization of the seminiferous tubules were observed, reflecting the absence of differentiating spermatocytes and spermatids. At the late pachytene stage, up to 90% of spermatocytes were dead. Furthermore, in the *Hsf2*^{-/-} pachytene spermatocytes, the synaptonemal complex, which forms an axis of paired chromosomes, was often disorganized showing an abnormal loop-like structure between pairs of homologous chromosomes.¹⁰ Disruption of both *Hsf1* and *Hsf2* caused a more severe phenotype associated with male sterility and a potentiation of the phenotype seen in *Hsf2*^{-/-} mice, suggesting that transcriptional activity of both factors is required for normal spermatogenesis.¹² Global expression analyses in testis of double knockout mice, demonstrated changes in expression patterns of genes involved in spermatogenesis.¹² Together these observations strongly suggest that the activities of HSF1 and HSF2 are tightly intertwined during spermatogenesis. Identification of the direct target genes is a prerequisite for understanding the physiological functions of HSFs in testis.

FUTURE PERSPECTIVES

Previously, HSFs were identified solely as regulators of *Hsps*, whereas now there is unambiguous evidence for HSFs having a great variety of target genes (TABLE 1.). In *S. cerevisiae* and *Drosophila*, about 3% of the genomic loci were identified as targets for HSF upon heat stress.^{57,58} 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 *Hsf* knockout mice have rendered the possibility of identifying novel targets. However, a challenging genome-wide ChIP-microarray approach to investigate *in vivo* targets of mammalian HSFs could uncover entirely novel gene clusters, pathways and functions for these transcription factors. This approach would most certainly broaden the current view of HSFs.

The functional relationship between different HSFs, both in cell stress and in developmental processes, is of great interest, and a novel dimension of the cooperation between HSFs is emerging. Synergy of DNA-binding activities among different transcription factors offers an efficient way to control gene expression in a cell- and stimulus-specific manner. By interacting with distinct partners and responding to both stress and developmental stimuli, HSFs could orchestrate differential gene regulation. It will be intriguing to elucidate whether HSF-mediated regulation depends on the activity of individual trimers, or whether homo- or heterotrimer formation is a common theme in HSF-mediated transcription (Fig. 2.). Obvious

questions for future studies are the stoichiometry between HSF1 and HSF2 in a possible complex and the mechanism by which the factors interact with each other. Given the slightly different binding preferences of HSF1 and HSF2,² the composition of the HSE on the target promoter could direct the formation of a specific heterocomplex. Sequence variations of the HSE, in a specific chromatin environment determined by histone modifications, could be an efficient way of regulating the DNA-binding ability of HSFs.

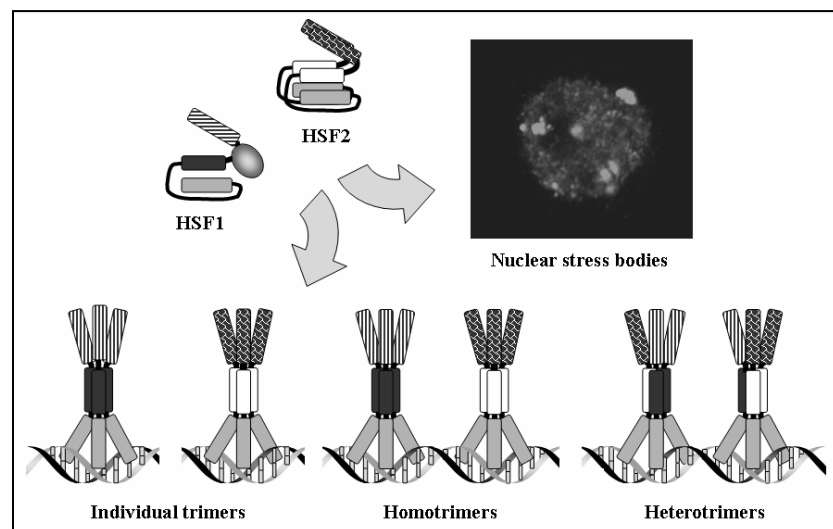


FIGURE 2. Activation and complex formation of HSF1 and HSF2 on the DNA. Inactive HSF1 is kept as a monomer, whereas HSF2 is a dimer. Upon activation HSFs trimerize and translocate to their target gene promoters and nuclear stress bodies (nSBs) mainly formed on locus 9q12 (confocal microscopy image is a courtesy of Anton Sandqvist). The possible composition of HSF trimers, either individual, or combined homo- and heterotrimers, on the DNA is displayed in the figure.

It is not exactly known how the cells sense stress. The correlation between longevity and stress resistance suggests that the ability to sense and respond to environmental challenges is important for the regulation of life span. Results from several groups indicate a direct role for HSF1 in the regulation of life span.^{42,43,59} Interestingly, down-regulation of HSF1 leads to both decreased life span and an accelerated aging phenotype in *C. elegans*. Recent discoveries demonstrate that a mutation conferring longevity also delays polyQ aggregation and toxicity, suggesting a link between the regulation of aging and aging-related diseases. Inactivation of *Daf-16*, *Hsf* or small *Hsps*, accelerates the aggregation of polyQ expansion proteins in *C.*

elegans.^{42,43} Correspondingly, human diploid fibroblasts show attenuated heat-inducible HSF1 DNA-binding activity and a decrease in Hsps upon aging.⁶⁰ These findings support a model where HSF1 is a key molecule for coupling the regulation of life span with the ability of cells to sense stress. Many pathologies in humans are associated with stress, age and expression of misfolded proteins, and several HSF-targeted therapeutic strategies have already been proposed. Small molecular regulators of the HSF activity have been identified and will be valuable tools for discovering novel therapies.⁶¹ The functional interplay between different mammalian HSFs emphasize that great consideration is required when planning future HSF-targeted therapies.

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