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The “HSF connection”: pleiotropic regulation and activities of Heat Shock Factors shape pathophysiological brain development

Agathe DUCHATEAU^{1,2,3,§}, Aurélie de THONEL^{1,2,§}, Rachid EL FATIMY^{1,2,*}, Véronique DUBREUIL^{1,2}, Valérie MEZGER^{1,2,&}

¹ Université de Paris, Epigenetics and Cell Fate, CNRS, F-75013 Paris, France

² Département Hospitalo-Universitaire DHU PROTECT, Paris, France.

³ ED 562 BioSPC, Université Paris Diderot Paris 7, F-75205 Paris Cedex 13, France

[§] These authors equally contributed to the study

& corresponding author : valerie.meger@univ-paris-diderot.fr

* present address: Department of Neurology, Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, 60 Fenwood Rd, 9006, Boston, MA, 02115, USA.

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Ethical issues

Hsf2^{+/+} (WT) and *Hsf2*^{-/-} from the *Hsf2*^{tm1Mmr} mouse strain on the *C57BL/6N* x *C57BL/6J* mixed background and the corresponding experimental protocols have received the agreement # 5314 from Ministère de l'Enseignement Supérieur et de la Recherche and were approved by the Institutional Animal Care and Use Ethical Committee of the Paris-Diderot University (registration number CEEA-40).

The authors declare no competing interests.

Abbreviations : CNS : Central Nervous System ; CP : cortical plate ; HS : Heat Shock ; HSE : Heat Shock Element ; HSFs : Heat Shock Factors ; Hsp : Heat Shock Protein ; MAP : Microtubule-associated Proteins ; NPC : neural progenitors cells ; PTMs : Post transcriptional modifications

Abstract

The Heat shock factors (HSFs) have been historically identified as a family of transcription factors that are activated and work in a stress-responsive manner, after exposure to a large variety of stimuli. However, they are also critical in normal conditions, in a life long manner, in a number of physiological processes that encompass gametogenesis, embryonic development and the integrity of adult organs and organisms. The importance of such roles is emphasized by the devastating impact of their deregulation on health, ranging from reproductive failure, neurodevelopmental disorders, cancer, and aging pathologies, including neurodegenerative disorders.

Here, we provide an overview of the delicate choreography of the regulation of HSFs during neurodevelopment, at prenatal and postnatal stages. The regulation of HSFs acts at multiple layers and steps, and comprises the control of (i) HSF mRNA and protein levels, (ii) HSF activity in terms of DNA-binding and transcription, (iii) HSF homo- and hetero-oligomerization capacities, and (iv) HSF combinatory set of post-translational modifications. We also describe how these regulatory mechanisms operate in the normal developing brain and how their perturbation impact neurodevelopment under prenatal or perinatal stress conditions. In addition, we put into perspective the possible role of HSFs in the evolution of the vertebrate brains and the importance of the HSF pathway in a large variety of neurodevelopmental disorders.

Keywords

Heat shock transcription factors, HSFs; neurodevelopment; prenatal stress; transcription; vertebrates

Introduction

Heat Shock Factors are a family of transcription factors characterized by their evolutionarily conserved stress-responsiveness and their ability to recognize a consensus Heat Shock Elements (HSE) DNA-binding site (Fig. 1). Historically, HSFs have been identified as drivers of the expression of genes encoding Heat Shock Proteins (HSPs), which are molecular chaperones that contribute to maintain protein homeostasis (proteostasis), under physiological and various stress conditions [1,2].

Their implication extends beyond the regulation of proteostasis, since HSFs do not only regulate *Hsp* genes, but also a broad repertoire of target genes, which are involved in a large spectrum of biological functions, such as development, gametogenesis or aging, notably *via* the regulation of translation, metabolism and stress adaptation [3–5]. The transcriptional regulation mediated by HSFs relies on their intrinsic transcriptional activity, but also on their abilities to remodel the epigenetic landscape, a process in which HSFs act by recruiting chromatin players on their target genes [5,6]. In light of this complexity, the deregulation of HSF activity has been linked to a large set of diseases including cancers, neurodevelopmental and neurodegenerative disorders.

HSF proteins are highly conserved across a large range of eukaryotes [7] and, in this review, we will mainly focus on vertebrate HSFs. In contrast to yeast, nematodes and insects, where a single HSF has been identified, vertebrates display a whole family of HSFs, of which HSF5, HSFX and HSFY seem specific to humans [3,5,8,9]. The versatile functions of HSFs have been mostly studied with HSF1 and HSF2, and to a lesser extent with HSF3 and HSF4 (reviewed in [3,5]). In mammals, HSF1 is the master regulator of the Heat Shock Response (HSR), thereby regulating, upon various proteotoxic stress, the protein quality control machinery expression, notably *via* the induction of HSPs. HSF1 also directs transcriptional programs that are uncoupled to the HSR, during development : HSF1 is a maternal factor essential for oogenesis, but also early preimplantation of the embryo, spermatogenesis, and olfactory neurogenesis ([10,11], reviewed in [3–5]). HSF2 also possesses developmental roles in early embryogenesis and gametogenesis (reviewed in [4,5]). In contrast to HSF1, HSF2 is responsive to chronic stresses of relevance for pathological situations, such as fever-like temperatures at 39-41°C [12], alcohol (ethanol) exposure [13,14], and prolonged proteasome inhibition [15–17]. The main function of HSF3 protein appears to be the regulation of the cellular HSR to severe and long-term stresses [9; 18–20]. Some developmental roles have also been proposed for HSF3 on the regulation of non-*Hsp* genes [21]. HSF4 plays a complex role in the development of lens and olfactory sensory placodes, with cooperative or antagonist effects with HSF1, operating on both *Hsp* and non-*Hsp* genes [22,23]. In that respect, the mutation of HSF4 in its DNA-binding domain (DBD) is implicated in congenital juvenile cataract formation [24].

HSF family members exhibit unanticipated complexity in their structure, DNA-binding selectivity, and post-translational modifications (PTMs), thereby governing their activity and interaction with a large variety of partners [5]. Several functional domains in HSF proteins are highly conserved between species (Fig.1A) [5,25-32]. In particular, all HSFs possess a N-terminal winged helix-turn-helix DBD, which is able to recognize and bind to the specific consensus HSE (Fig. 1C), defined as a succession of several inverted repeats of the pentanucleotide motif nGAAn ([33,-38], reviewed in [5]). Another conserved domain is the N-terminal oligomerization domain (HR-A/B), which is composed of three hydrophobic heptad repeats and allows the formation of a triple coiled-coil between three HSF monomers. The HR-A/B domain is required for the formation of HSFs homo- or heterotrimers, which represent the DNA-binding HSF conformations [39,40]. In all HSFs, except HSF4, there is another hydrophobic heptad repeats domain (HR-C) located at the C-terminus of the protein (Fig. 1B), which inhibits trimerization by maintaining the inactive monomeric conformation [26]. The transcriptional activation of HSFs requires the activation domain (AD), a domain of interaction with cofactors, which is negatively regulated by the regulatory domain (RD) that is itself controlled by numerous post-translational modifications (PTMs) [39-44]. Therefore, the DNA-binding of HSFs trimers does not necessarily induce the expression of target genes [45].

In physiological conditions, HSF1 is maintained in a monomeric conformation by intra-molecular (HR-A/B and HR-C domain) and intermolecular interactions (with HSP70, HSP90 and TRiC (cytosolic chaperonin TCP1 ring complex [46]). Upon stress, like heat shock (HS), HSF1 is activated and then deactivated *via* a multistep cycle, which is highly regulated by a complex combination of PTMs; the latent inactive HSF1 monomers form trimers, thanks to the oligomerization domain (HR-A/B, Fig. 1C), that is translocated into the nucleus. Similar to HSF1, HSF2 is maintained as an inactive monomer but also as a dimer (as is also HSF3), becomes trimeric and acquires DNA-binding activity by still unknown mechanisms [8,13,47]. In HSF4, the lack of HR-C domain results in its constitutive trimerization and DNA-binding ability [9]. The HR-A/B domain is surrounded by two putative nuclear localization sequences (NLS), NLS1 and NLS2, both of which are required for the nuclear translocation of HSF2 [48]. In contrast, only the equivalent of NLS2 is needed for HSF1 and HSF3 nuclear translocation [49,50]. HSFs can be subjected to differential regulation by protein-protein interactions and PTMs. In this respect, HSFs can act both as activators and repressors depending on the environmental conditions and the developmental stages [35,38]. In addition, subtle crosstalks exist between HSFs, which, together with the combinatorial possibilities, allowed by the different PTMs, multiply the levels of fine-tuning HSFs target genes. For instance, HSF1 can form heterotrimers with HSF3 to regulate the HSR, at least in lizards [9], or with HSF2, which acts as a fine modulator of the HSR mainly driven by HSF1 [39]. HSF1 and HSF4 can also act in a cooperative or antagonistic manner during lens

development [22]. Furthermore, while all HSF members share the ability to bind the consensus HSE, they exhibit differential preferences in terms of the recognition stringency for the HSE consensus, the architecture (number of required inverted repeats of the pentanucleotide motif), and localization of HSEs in promoter, intergenic or intronic regions [51,52]. These binding specificities vary among tissues, developmental stages, and environmental contexts and participate in the subtle diversity of HSF target genes and the complexity of their regulation [33,53].

The regulation of HSF1 stability and activity is strongly shaped by PTMs, such as phosphorylation, sumoylation, and acetylation, having either activating or inhibiting effects, and this in a stimulus-dependent manner [54–57]. Several kinases of importance for brain development and adult brain integrity are involved. In the absence of stress, the phosphorylation of HSF1 is primed by kinases like ERK1, and leads to its ubiquitin-mediated degradation [57]. A member of the casein kinase 2 (CK2) family also controls HSF1 proteasomal degradation, with key impact in Huntington disease [7]. Playing on another layer of regulation, MEK1, a protein kinase of the oncogenic RAS/MAPK pathway, promotes HSF1 nuclear translocation and activity during the HSR, while ERK1/2 kinases indirectly counteract HSF1 activation *via* a negative feedback mechanism [58]. Phosphomutants of HSF1 do not oligomerize and, HSF1 hyperphosphorylation impairs the transcription of target genes whose promoters contain an HSE bearing only three nGAAn units, and not more [59]. However, the hyperphosphorylation of HSF1 in the RD domain does not impact HSF1 subcellular localization and DNA-binding activity of HSF1 [60]. PKA, which phosphorylates HSF1, is essential for the effective transcription of *Hsp* genes upon HS, by triggering the binding of HSF1 to the histone/lysine acetyltransferase EP300 [61,62]. EP300/CBP also acetylate HSF1, and are thus key modulators of the HSR [56,63]. Indeed, a complex balance between acetylation and deacetylation of HSF1 by histone/lysine acetyltransferase EP300 and deacetylases (SIRT1, HDAC7 and HDAC9) regulates its DNA-binding activity ([56,63–65], reviewed in [6]). Moreover, acetylation by EP300, but not CBP, seems also required for HSF1 stabilization in the HSR [56]. Of note, no regulation of HSF2 by phosphorylation has been reported so far.

HSF1, HSF2, and HSF4 can be sumoylated, but in different domains and with different effects. HSF1 is sumoylated on several lysines located in the RD domain, which leads to inhibition of its transactivation capacity, at a magnitude dependent on the severity of the stress. Namely, the most severe the HS is, the more HSF1 becomes sumoylated and rapidly inactivated [66,67]. In contrast, the sumoylation of HSF2 occurs in the DBD and leads to the inhibition of its DNA-binding capacity, without affecting its oligomerization [68,69].

HSF2 is a very labile protein [70,71] whose levels fluctuate and exhibit marked dependency on cellular contexts and phases of the cell cycle [72]. The amounts of HSF2 proteins must indeed be highly

regulated and their stabilization is an important control step in mediating HSF2 physiological role and in the tuning of the stress response. In that sense, during HS, HSF2 activation is tightly controlled by proteasomal degradation [73], which might explain its transient role in the induction of *Hsp* genes, and thus in the modulation of the HSR [39]. Recently, we showed that HSF2 stability is also dependent on its acetylation mediated by EP300/CBP [74].

In this review, we will focus on the status and roles of HSF1 to HSF4 in vertebrate brain development, knowing that most of the data have been produced in rodents.

Dynamic of the expression levels and patterns of HSFs along neural development

HSFs gene expression profiles

In the developing brain, the expression of the members of the HSF family, varies among the vertebrate species, which is suggestive of divergent mechanisms of gene regulation, and further of a diversity of functions. In the zebrafish, *zhsf1* and *zhsf2* mRNAs display similar expression patterns at various stages, suggesting common mechanisms of regulation [75]. In contrast, in the chicken the three cHSF proteins, which are ubiquitously expressed in the embryonic brain from day 3 to day 12 [20], show uncorrelated levels of expression: *HSF2* (*cHSF2*) transcripts are strongly expressed at all stages from embryonic day 4 to day 12, whereas *cHSF1* and *cHSF3* mRNAs are detected at low levels [21].

In the mouse, in contrast with *mHSF4* mRNAs, which are detected at very low levels in the developing central nervous system (CNS), but accumulate postnatally (Fig. 2A), *mHSF1* and *mHSF2* genes are both expressed during the prenatal and postnatal brain development. However, they exhibit very dynamic and distinct profiles of expression and lead to marked differences in HSF1 and HSF2 protein activity. *mHsf2* transcripts are expressed in the whole CNS at early stages (E9.5-E13.5), and then are restricted to the brain [76-78]. RNA-Seq data show that *mHSF1* mRNA levels are detected between E11.5 and E18 in the cortex (Fig. 2A) and are distributed homogeneously in the dividing neural progenitors (NPC) compartment and in the postmitotic young neurons of the developing cortical plate (CP; at least at E14; [13]). *mHsf2* transcripts are detected at much lower levels in RNA-seq data, compared with *mHSF1* (Fig. 2A), and are concentrated in the ventricular or subventricular zones, which contains NPCs, being almost absent from the first layers of young postmitotic neurons of the CP until the E14 stage ([13]; Fig. 3A). However, from E15, *mHsf2* mRNAs are detected in the latest born neurons, which populate the two most external neuronal layers of the CP (layers II and III; [13,78]; Fig. 3A). It seems therefore that *mHsf1* and *mHsf2* transcriptional (and/or post-transcriptional) regulation are distinct along the cortical development.

In the prenatal developing cortex, mHSF1 and mHSF2 proteins are detected at all stages (Fig. 4A and 5, [13,77–79]). The levels of mHSF2 protein, elevated at early stages of cortical development up to E13, and decreasing progressively until E18, roughly parallel the abundance of their transcripts (Fig. 2A and 5, [13,76]), suggesting they are governed at the transcriptional level during the developmental period. The presence of a conserved E-box in the regulatory region of the *Hsf2* gene in mice, rats and humans and of USF, a major E-box-binding protein in the brain, might contribute to this pattern [80–82]. In addition, since the *mHsf2* transcripts are expressed at relatively low levels (Fig. 2A), and since HSF2 protein has a rapid turnover, its elevated levels in the prenatal developing cortex might also depend on its stabilization against proteasomal degradation [70,71,73,74,83].

In the postnatal brain, rodent HSF1 and HSF2 proteins also show very different dynamics. At the perinatal period, low HSF1 and HSF2 levels in the cortex are observed (Fig. 5, [84,85]). They further increase progressively in the murine and rat cortex [Fig. 5, [82,84,85]] (summarized in Fig. 4A, in the case of mouse and Fig. 5). In the early postnatal cerebellum, rat HSF1 (rHSF1) protein levels are very low, but start to increase at P10, and peak at P30. On the opposite, rHSF2 proteins are detected at P0 and P10, but show decreased levels at P15 and P30. Low levels of rHSF1 are also detected in the brainstem and similarly increase at later stages, with a peak between P20 and P30 [84,85].

Little data is available in humans, but the recent development of human brain organoids that recapitulate early stages of cortical development in terms of neural markers, cell diversity and structure represents a growing source of data [86,87]. We and others have shown that *hHSF1* and *hHSF2* transcripts, as well as *hHSF1* and *hHSF2* proteins, are found in human brain organoids [74,88]. These results are in line with the detection of *hHSF2* transcripts in RNA-Seq data in different regions of the fetal brain between post-conception weeks 8 and 16 (Fig. 2B), whereas *hHSF1* and *hHSF4* mRNAs are almost undetectable (data not shown). RNA-Seq data generated from human adult brains show that *hHSF1* mRNAs are expressed in different brain regions, but at lower levels than in other organs, whereas in the mouse, *mHsf1* RNA are detected at levels similar in the adult brain as in other organs (Fig. 2A and 2C). *hHSF2* and *hHSF4* transcripts are more abundant in the cerebellum than in other brain regions, which is not the case in the mouse (Fig. 2A and 2C). These data are altogether suggestive of species differences in terms of HSF gene transcriptional regulation or mRNA stability.

HSF protein localization and activity

The nuclear localization of cHSF2 in cells of neural origin, as well as its high expression levels in the chicken developing brain have supported the notion that, in contrast to cHSF1 or cHSF3, cHSF2 might be specifically involved in the chicken neurodevelopment [20]. Similarly, mHSF2 seems mainly nuclear throughout prenatal cortical development [13,77,78] (Fig. 3B, 4A). In contrast, mHSF1 is

majorly cytoplasmic, in the prenatal cortex, with some young neurons of the CP showing nuclear localization [13,89] (Fig. 3C, 4A). In the postnatal brain, notably at P30, rHSF1 is localized in the nucleus of neurons and oligodendrocytes of different brain regions, including the cerebellum, brainstem and cortex. In contrast, rHSF2 is then majorly perinuclear, cytoplasmic, and present in the dendritic processes of neurons, in the same regions [84,85]. Rodent HSF1 and HSF2 proteins therefore display distinct patterns of subcellular localization in the pre- and postnatal cortex [13,89].

Interestingly, hHSF2 is mainly localized in the nucleus of both NPCs and young postmitotic neurons in day-60 organoids, a stage equivalent to early-mid neurogenesis. This might suggest that HSF2 in humans is present in some postmitotic neurons at earlier stages of neurogenesis compared to mice [74].

The DNA-binding properties of HSF1 and HSF2 also considerably differ along the prenatal and postnatal brain development and parallel their nuclear localization. In chicken, cHSF2 proteins show DNA-binding activity at very early embryonic stages (Fig. 6). In the same line, mHSF2 also displays spontaneous DNA-binding activity throughout cortical development, as indicated by *ex vivo* (gel shift assay, Fig. 4A, 7A and 7B) and *in vivo* experiments (chromatin-immunoprecipitation (ChIP) experiments; [13,76–78]). In contrast, mHSF1 although presents throughout the prenatal cortical development, does not spontaneously bind DNA (HSE) in the fetal cortex according to *ex vivo* experiments ([13,76–78], Fig. 4, 7A and 7B). Therefore, only mHSF2 homotrimers can be detected both in the developing cortex and in neural cell systems ([13,76,78], Fig. 4B). This spontaneous DNA-binding activity of mHSF2 in the prenatal cortex is strongly suggestive of specific properties and function in development. mHSF4 does not seem to contribute to HSF DNA-binding activity neither prenatally nor postnatally in the cortex, or the cerebellum, even though *mHSF4* transcripts and protein are detected ([90], Fig. 2A, 7A and 7B, and VM unpublished results). Indeed, HSF4b displays neuronal expression in the adult mouse brain, including the cerebellum, but it is co-expressed with the phosphatase DUSP26, which can reduce its DNA-binding abilities by counteracting its phosphorylation by MEK1 [90].

After birth, there is an almost complete loss of HSF DNA-binding activity in the mouse cortex (Fig. 4A and 7A), although mHSF1 protein is still detected in the hippocampus at P2 [91]. From P12 to P22, HSF DNA-binding activity is observed, to which both mHSF1 and mHSF2 contribute (Fig. 7A and 7B), and is suggestive of the spontaneous formation of heterotrimers in the cortex at these stages (Fig. 4B). This contrasts with the situation observed in older animals (P210), in which the HSF DNA-binding activity is only due to mHSF1, but not to mHSF2 (Fig. 7B and 7C; see below). Whether the striking dynamics of HSF1 and HSF2 DNA-binding activity as homo- or heterotrimers along the postnatal, young adult and adult period, are regulated at the transcriptional, post-transcriptional, or

post-translational levels is still to be determined. The fact that similar levels of *mHsf1* mRNAs are observed before birth and in the adult, albeit with such differences in term of DNA-binding activities, is again suggestive of additional levels of régulation (Fig. 2A and 7C).

The control of HSF1 and HSF2 protein levels and activities is known to rely on PTMs and it is also potentially the case along cortical development. Indeed, the abundance of mHSF2 and hHSF2, which are short-lived proteins, is correlated with the acetylation of these proteins in the cortex and in human brain organoids, a PTM that reduces HSF2 proteasomal degradation [13,74]. In basal conditions, in the neural context, mHSF1 is found acetylated and also slightly sumoylated, which might explain part of its inactivity [13]. So far, nothing is known about the DNA-binding activities of human HSFs in organoids or brains.

There is no formal demonstration that alternative splicing events, as described in other, non-neural, systems (reviewed in [5]), contribute to the diversity of HSF forms in the brain, except for HSF4b [90,92]. However, the detection of different forms of HSF1 and HSF2 proteins in the pre- and postnatal brain with various molecular weight is compatible with such hypothesis, as it is the case for HSF2 with its two isoforms α and β (e.g. [76]). Because HSF isoforms can have different properties in terms of transcriptional activation, their diversity could even render the HSFs landscape and the HSFs combinatory modes of action even more subtle (reviewed in [5]).

HSF protein localization and activity upon insults in neural cells

In response to various embryonic (*ex vivo* heat shock) or prenatal insults, including maternal epilepsy, exposure to heavy metals or alcohol, a translocation of rodent HSF1 into the nucleus is observed in NPCs and young neurons, but not in mature neurons, thereby indicating that this process exhibits cell-type specificity [13,79,89]. In parallel, HSF1 DNA-binding capacities are activated (both *ex vivo* in gel shift assays, or *in vivo* in ChIP experiments) together with its transcriptional activity (RT-qPCR or microarrays; [13,76-79,89,93] ; Fig. 4A, 5 and 7). The activation of HSF1 by ethanol in the developing cortex and in neural cells strikingly depends on the presence of HSF2 [13]. Alcohol exposure triggers the formation of atypical HSF1-HSF2 heterotrimers, in comparison to those induced in response to heat shock in neural cell models. These heterotrimers play a detrimental role in the developing cortex, altering the expression levels of genes involved in radial neuronal migration, that are regulated by HSF2 homotrimers in normal conditions. This occurs in two ways: either the formation of HSF1-HSF2 heterotrimers prevents binding to the HSE or it replaces HSF2 homotrimers [13]. This suggests that mHSF2 has specific properties and roles in the prenatal cortex, likely linked to its ability to bind DNA spontaneously. It seems that HSF1 DNA-binding activity in response to insults does not depend on its protein level *per se*, but rather on the ratio between the two HSFs, HSF1 and

HSF2. Indeed, in the developing cortex or in neural progenitor cell models, which contain a high HSF2/HSF1 ratio, the activation of HSF1 in response to alcohol exposure is dependent on HSF2, whereas it is not the case in cells containing a low HSF2/HSF1 ratio [13].

Specific PTMs are associated with the unconventional properties of HSF1 activation by ethanol in the prenatal cortex. The absence of HSF1 hyperphosphorylation, a hallmark of HSF1 activation in response to heat shock, signs the specificity of the mechanisms elicited by ethanol in the developing cortex. The elevated sumoylation levels of HSF1, which are classically observed in response to sustained heat shock, participate to its rapid inactivation. In contrast, the modest levels of HSF1 sumoylation observed in the alcohol-exposed developing cortex in sustained, chronic manner, likely confer this factor ability to remain persistently active [13].

Roles of HSF1 and HSF2 in the normal developing prenatal and postnatal cortex

The first functional evidence for a role of HSFs in the developing cortex came from studies in *Hsf2* knockout (KO) mice ([77,78], reviewed in [4]). Adult *Hsf2* KO mice display neurodevelopmental defects characterized by enlarged ventricles, reduced hippocampus size and cortex width [94]; *Hsf2*^{tm1Miv} [129S2/SvPasx129X1/SvJxC57BL/6 genetic backgrounds]; *Hsf2*^{tm1Mmr} [77] [C57/Bl6N genetic background]; [78] [C57/Bl6N genetic background]; [13] [mixed C57/Bl6N x C57/Bl6J] genetic background). In line with these defects, ventricular collapse and intracerebral hemorrhages were detected in *Hsf2*^{tm1Miv / tm1Miv} embryos. Adult brain structural defects were not observed in another *Hsf2* KO mouse strain ([95]; *Hsf2*^{tm1Ijb} [129S6/SvEvTac x 129X1/SvJ] or [129S6/SvEvTac x C57BL/6] genetic background). This might be due to differences in the mouse genetic background (see also [4]). Accordingly, we found that the penetrance of the neurodevelopmental phenotype of the disruption of the *Hsf2* gene (*Hsf2*^{tm1Miv / tm1Miv}) was more severe in the C57Bl/6N [77,78] than in the C57Bl/6N x C57Bl/6J genetic background [13]. It can also be due to the fact that McMillan *et al.* only reported data on the adult brain, which could mask some subtle neurodevelopmental phenotypes [95].

We further showed that HSF2 controls, at multiple levels, the radial neuronal migration process that underlies the establishment of the neocortex neuronal layers during the prenatal mouse development [78,96]. First, the lack of HSF2 in the developing cortex affects two cell types that assist radial neuronal migration: the Cajal-Retzius cells and the radial glia. HSF2 controls the number of Cajal-Retzius cells, which are located in the marginal zone (at the surface of the neocortex), and secrete Reelin, a key signaling molecule for the migration of cortical neurons. As a consequence, in *Hsf2*^{tm1Mmr / tm1Mmr}, reduced levels of Reelin and of its downstream target - the phosphorylated form of Dab-1, located in migrating neurons - are observed. The lack of HSF2 also results in a lower number of radial glia fibers, which serve as architectural guides for the migration of young neurons. Second, HSF2 plays an intrinsic

role in postmitotic neurons themselves, as observed by the specific defective radial neuronal migration of the neurons that express HSF2: the layer II-III neurons, whereas neurons from the layers VI-IV, which do not express HSF2 are unaffected. HSF2 controls neuronal migration by regulating the expression of genes that govern the dynamics of microtubules, such as the microtubule-associated proteins (MAPs; [13,78,96]): members of the Doublecortin family (Doublecortin, DCX and Doublecortin-like kinase 1, DCLK1), MAPT (Tau; [96,97]), an unconventional myosin family member, myosin 10, which interacts directly with and regulates both microfilaments and microtubules (MYO10; [98]), as well as a kinase (MARK2) and two activators of the Cdk5 kinase (p35 and p39), that regulate doublecortin and radial neuronal migration [96,99,100].

Beside these migration defects, the reduction in radial glia cells in the absence of HSF2, is also suggestive of abnormal proliferation [78]. This hypothesis is supported by the observed deregulation of genes involved in the cell cycle upon *Hsf2* gene inactivation [94] and the many *MAP* genes targeted by HSF2, known to be involved in the control of proliferation [96,101–103].

HSF2 regulates also genes involved in cell adhesion. For example, CHL1, a member of the L1 family of cell adhesion molecules (CAMs), controls neuronal positioning in the developing cortex [104] and is downregulated in the absence of HSF2 [13]. Recently, the repertoire of HSF2 target genes has been enriched by the identification of genes encoding the Cadherin superfamily of cell-cell adhesion proteins [17]. This suggests that the role of HSF2 could be also mediated by this new family players, and extended to various levels of neurodevelopment, which control multiple aspects of brain formation and integrity, including the reelin pathway [105], neuronal migration *per se* [106,107], and synaptic differentiation and activity [108,109].

The study of *Hsf1* KO mice has brought no evidence that HSF1 might be involved in prenatal neurodevelopment [89]. However, it has been involved in the early postnatal development which, in humans, corresponds to the third trimester of pregnancy. Early hippocampal development depends on HSF1 and, in adult, *Hsf1* KO (*Hsf1*^{tm1Anak / tm1Anak}) displayed reduction in dendritic length and spine density in neurons of the hippocampus [91]. Neuronal density is unaltered, but adult neurogenesis is reduced in the KO and associated with premature but incomplete neuronal differentiation in the dentate gyrus. Altogether, these data are indicative of impaired maturation of the dentate gyrus [86]. In conjunction with these defects, adult *Hsf1* KO mice exhibit disturbed affective behavior, in terms of anxiety and sociability, increased aggressiveness and depression-like behavior. The abnormal depressive and anxiety behavior can be rescued by overexpressing a constitutive form of mHSF1 in the neonatal hippocampus (P2), but not in the adult. This rescue was correlated with a restoration of the expression levels of PSD95, a postsynaptic protein marker. In link with these features, decreased

levels of polysialic acid-neural cell adhesion molecule (PSA-NCAM), which promotes synaptogenesis and plasticity, were also detected in *Hsf1* KO neonates. This correlates with impaired HSF1-dependent transcription of two genes implicated in the biogenesis of PSA, the polysialyl-transferases, *ST8SialI* and *ST8SialIV* [91].

Hsf1^{tm1Anak / tm1Anak} adult mice also showed enlarged lateral ventricles, as do *Hsf2*^{tm1ljb / tm1ljb} [110]. This enlargement of ventricles could be due to the role of HSF1 in the maintenance of ciliary beating, which could result in abnormal circulation of the cerebral spinal fluid and hydrocephaly [111]. Crucial roles of HSF1 and HSF4, unraveled in other systems, on DNA damage/repair and anti-apoptotic pathways, as well as of HSF4 on lysosomal pathways, could be also at play in neurodevelopment or brain integrity [112–114]. Finally, astrogliosis, demyelination, accumulation of poly-ubiquitinated proteins and neurodegeneration were also found in *Hsf1* KO mice indicating its role in postnatal neurogenesis and/or maintenance of neuronal homeostasis rather than a role in prenatal development of the brain [110,115].

To our knowledge, there is no data available on neurodevelopmental phenotypes in *Hsf4* KO mouse models (*Hsf4*^{tm1Anak} [22]; *Hsf4*^{tm1Miv} [23]; and *Hsf4*^{tm1Xyk} [116]), and no *mHSF4* mRNAs or DNA-binding activity could be detected in the fetal cortex (Fig. 2A and 7).

Intricacy between the stress-responsive and neurodevelopmental roles of HSFs

In a counter-intuitive manner, although constitutively possessing DNA-binding and transcriptional activities during whole cortical development, as demonstrated for the above-cited neurodevelopmental genes, HSF2 does not seem to control the expression of many *Hsp* genes in normal conditions: the profiles of expression of HSF2 and HSPs transcripts and proteins, and of a reporter gene under the control of the *Hsp70.1* promoter are distinct prenatally and postnatally [76,84]. Moreover, there is no obvious alteration in the levels of the main HSPs in *Hsf2* KO mice (*Hsf2*^{tm1Mmr / tm1Mmr}; [94]). Accordingly, HSF2 does not bind the regulatory region of the *Hsp70* gene in the E16.5 cortex *in vivo* [13]; although it binds that of *Hsp90*). In line with its lack of DNA-binding activity, HSF1 does not either bind *Hsp70* or *Hsp90* regulatory regions in the developing cortex in normal conditions. In contrast to developing period, in the adult brain, Hsp27 and CRYAB levels are deregulated in specific brain regions of *Hsf1* KO mice, and might be responsible for increased cell death. The accumulation of HSP27, CRYAB, and polyubiquitinated proteins represents hallmarks of stress, which might be correlated with astrogliosis and demyelination [110,115] (NB: the role of disruption of the HSF pathway in neurodegeneration is reviewed in [5]).

In striking contrast, in response to prenatal ethanol exposure in models of Fetal Alcohol Syndrome (FAS), both mHSF1 and mHSF2 bind to the *Hsp70* and *Hsp90* promoter region and drive

their expression, in the developing cortex [16,89,93]. Human fetal cortical samples exposed *ex vivo* to ethanol also show the upregulation of various HSPs [93]. In the mouse prenatal cortex exposed to alcohol either in chronic or binge-drinking-like manner, this occurs through the formation of ethanol-specific HSF1-HSF2 heterotrimers, as described above [13]. HSF1 also induces the expression of the *Hsp70* gene by binding to its regulatory region, in the prenatal cortex, in response to other prenatal insults: exposure to heavy metals (methylmercury - MeHg) or induced maternal seizures [93]. To date, no available data concerning HSF2 and these stressors has been reported. An important function of HSF1 in the prenatal brain is therefore to respond to environmental insults, as exemplified by the sensitivity of *Hsf1* KO mice to these insults, compared to heterozygous (or wild type) mice (*Hsf1*^{tm1Anak}; [89,117]): stressed *Hsf1* KO brains show decreased weight, heterotopia (signs of abnormal neuronal migration in the fetal cortex), cell cycle defects, apoptosis, and juveniles present with seizures compared to wild-type ones. The overexpression of mHSF1 can mitigate apoptotic cell death induced by prenatal ethanol exposure, as well as cell cycle defects. In contrast, HSP70 overexpression reduces apoptosis, but not cell cycle defects, suggesting compensation by either other *Hsp* or by non-*Hsp* target genes in that case. Non-*Hsp* genes, like *MAP* genes or genes of the Cadherin superfamily, could be attractive candidates.

Interestingly, the prenatal brain displays a very specific HSF context, because it contains high physiological levels of HSF2 that correlate with the presence of homotrimers of HSF2, constitutively active for DNA-binding. Upon ethanolic stress, mHSF1 and mHSF2 form heterotrimers leading to three situations in the regulatory regions of HSF2 target genes: 1) regions not bound by HSF2 gain binding by the HSF1-HSF2 heterotrimers, as observed in *Hsp70* gene; 2) regions bound by HSF2, become occupied by HSF1-HSF2 heterotrimers, (e.g. *Hsp90* and the *MAP* gene *Dclk1*), and 3) regions bound by HSF2, are not occupied anymore, as is the case for other *MAP* genes, *p35* and *Dcx* [13]. The alteration of gene expression induced by ethanol and mediated by the binding of the HSF1-HSF2 heterotrimers is beneficial in the case of *Hsp70* induction (notably, through the reduction of cell death), but detrimental in the case of the global reduction of expression of the *MAP* genes, which leads to neuronal migration defects. HSFs play therefore dual roles in neurodevelopment by regulating genes involved in the stress response, on the one hand, and in brain development, on the other hand. This might account for the double-edged sword effect of the HSF pathway when the developing brain is aggressed.

This dual role of HSFs in the developing brain might partly extend to differentiated neurons or the adult brain. Indeed, HSF1 activation was also observed in *ex vivo* cultured neurons treated with ethanol [118] and among the deregulated genes, the authors identified *Mtap1b*, encoding a microtubule associated protein, and *Cadherin 13* (*Cdh13*), members of the two major families of HSF2

non-*Hsp* target genes. The same authors [119] also identified HSF1 activation and induction of *Hsp* genes in primary culture of astrocytes, as well as many non-*Hsp* genes, including genes regulating transcription, cell proliferation and differentiation and more generally cytoskeleton networks, inflammatory/immune response, as well as metabolism, all of which could contribute to the brain vulnerability to alcohol, if deregulated in *Hsf1* KO mice.

Emerging roles of the HSF pathway in exacerbated cell-to-cell variability

The study of the roles of the HSF pathway in the normal or pathological development of the brain has also allowed the emergence of a key concept. Indeed, high and unexplained variability in terms of severity of brain abnormalities is observed in response to prenatal insults, whatever the dose, time-window and mode of exposure. The Hashimoto-Torii laboratory [120], has made a real progress in this field. They demonstrated that the activation of HSF1 in the developing brain exposed to ethanol occurs in a mosaic manner among the cells. This highly variable and stochastic activation impacts cortical development. Indeed, cells into which the activation of HSF1 is exacerbated, are those which exhibit the most disturbed neural cell fate, in particular concerning their migration and positioning into the appropriate cortical layer [120]. Those defects could thus be due to the deregulation of HSF1 target gene expression, including *Hsp* and non-*Hsp* genes, as stated above. Similarly, the existence of cell-to-cell variability, linked to the aberrant variability of HSF1 activation, was observed in patients with a neuropsychiatric disorder. Increased risk for schizophrenia (SZ) or autism spectrum disorders (ASD) is believed to have both genetic and environmental contributions. Both disorders have a neurodevelopmental component, which relies on a complex genetic architecture, based on a constellation of genetic variants or mutations [121], and meta-analyses have linked SZ to actors of the HSF pathway [122–124]. In addition, SZ and ASD also arise from prenatal exposure to viral infection, hypoxia, or systemic inflammation that can activate the HSF pathway [121,125,126]. Accordingly, heat shock at fever-range temperatures was shown to deregulate susceptibility genes for SZ and ASD in human induced pluripotent stem cells (iPSCs) [127]. In that context, Hashimoto-Torii *et al.* show that NPCs differentiated from SZ patient-derived iPSCs, display exacerbated variability in terms of induction of the *HSP70* gene, in response to ethanol or MeHg exposure [89].

Conclusion

Multiple and intricate levels of regulation, from transcription to post-translation, are therefore at play to provide the proper ratio of HSF1 and HSF2 proteins (and HSF3, in the case of chicken) and support the subtle control of their DNA-binding activities in one given species and one given embryonic stage. They also contribute to their ability to form homo- or heterotrimers and to be post-

translationally modified. The molecular mechanisms governing the regulation of these different steps and their crosstalks are still largely unknown and remain to be explored, but the sophistication of these expression and activity profiles are suggestive of important functions in the developing brain. Among these vertebrate species, the variety of expression and DNA-binding profiles of each of the HSF members, suggests that the role of the HSF pathway has evolved to fit the developmental constraints along the brain evolution in vertebrates. In this regard, HSF2 is exemplary. Although mHSF2 is strictly restricted to NPCs in the first stages of neurogenesis, and absent from the four deeper layers of the neocortex, it is expressed, at later stages, in the migrating neurons that form the two most superficial layers (II and III) in the neocortex, and finely tunes their migration [78] - the evolutionary expansion of which, correlates with increased callosal projections and increased neuronal diversity in mammals [128]. The evolutive recruitment of HSF2 in these neuronal populations, described to be under specific temporal regulation [129], might account for the variability of migrating behaviors and participate to neuronal diversity. Consequently, a double-edged effects of HSF2 is revealed in preclinical studies of FAS: HSF2, by driving HSF1 activation, both protects the young neurons by *Hsp70* gene activation [13,89], and perturbs their migration by failing to activate appropriately MAP genes. This might reflect an evolutionary “cost” based on the intricacy between the so-called classical roles of HSFs on *Hsp* gene expression and a more recently acquired role based on a *non-HSP* gene repertoire.

Abnormalities or disruption of the HSF pathway have been associated with neuropsychiatric disorders of neurodevelopmental origin, due to exposure to prenatal insults. Recently, alterations of the HSF pathway were identified in the rare monogenic neurodevelopmental disorder, Rubinstein-Taybi Syndrome [74], and also pointed out in Down syndrome [130]. Therefore, there is a possibility that the impairment of the pleiotropic effects of HSFs in regulating neurodevelopment, due to either environmental adversity or genetic origin, could account for a diversity of neurodevelopmental diseases. Interestingly, the presence of one mutation can have multiple detrimental effects on the proteome, thereby compromising proteostasis and modifying the physiological thresholds of HSF activation. In such cases, the origin of proteome imbalance could be: 1) variations in gene dosage (like in Down Syndrome) that deeply perturbs the formation of multiprotein complexes with deleterious consequences at the level of enzymatic activities or transcriptional regulation [130–132]; 2) alteration of the “chaperome” (the totality of molecular chaperones that, in the cell, ensure protein quality control caused by the presence of a single mutation) [133,134]. Indeed, either environmental challenge or the appearance of a cryptic mutation in a given genetic background, by reducing the amount of available chaperones, or by hijacking key chaperones (like HSP90) from their numerous physiological clients, can alter the global balance of the quality control of protein folding and increase

the load of misfolded proteins in the cell [135,136]. The resulting global alteration of the balance between the quality control of protein folding and the load of misfolded proteins could therefore generate impairment of the HSF pathway activation, either exacerbated or reduced in different cell subpopulations, and account for a larger number of neurodevelopmental disorders than has been previously anticipated. The development of patient-derived iPSC models that serve at modeling pathologies, coupled with the generation of brain organoids, and combined with new tools derived from the genome-editing and single-cell “omic” analyses, will likely generate exciting breakthroughs, in the future, in our understanding of the role of HSFs in physiological and pathological brain development.

Legends of Figures

Figure 1. Schematic representation of the typical, HSF protein domains and positioning of the HSF trimer on its consensus HSE site on DNA.

(A) Conserved HSF protein domains, based on that of human and murine HSF1. **DBD**: DNA binding domain which binds the consensus HSE; **HR-A/B** oligomerization domain: heptad repeat A (HR-A) and heptad repeat B (HR-B) leucine-zipper domains mediate the formation of triple coiled-coil trimeric HSF. **HR-C** heptad repeat domain, presents in all HSFs, except *S. cerevisiae* HSF and mammalian HSF4, maintains HSF in a monomeric form, thanks to its interaction with the HR-A/B domain. **AD** domain is responsible for transcriptional activation; **RD**: the regulatory domain that modulates AD activity. **(B)** Monomeric HSF conformation, unable to bind DNA. **(C)** DNA-binding of the trimeric HSF conformation, mediated by DBDs on the consensus HSE sequence. The HSE is composed of three inverted repeats of the pentanucleotide nGAAn (n denotes any nucleotide). HSE logo sequence obtained from the HOMER (v4.9.1) motif database, shaped with seqLogo R packages, based on the position weight matrix (PWM). Adapted from Wu [28].

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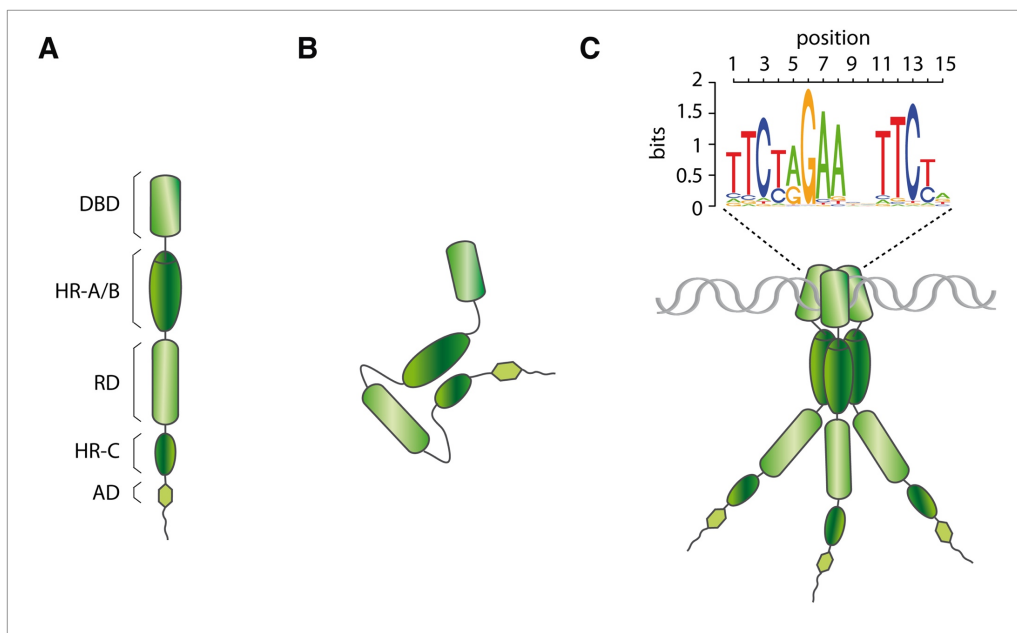
Figure 1

Figure 2. Gene expression of mouse and human *HSF1*, *HSF2* and *HSF4* in different tissues.

(A) Mouse gene expression of *HSF1*, *HSF2* and *HSF4* selected from the Mouse ENCODE transcriptome project in NCBI database (BioProject [PRJNA66167](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA66167)). Expression values are reported in Reads Per Kilobase Million (RPKM). All tissues are represented by one sample. P0 dashed line delimits embryonic (left) and adult (right) expressions. **CNS** : Central Nervous System. **(B) Embryonic human gene expression of *HSF2*** from Expression Atlas database (<https://www.ebi.ac.uk/gxa/home>). Values are expressed in Transcripts Per Kilobase Million (TPM). **(C) Adult human gene expression** of *Hsf1* (ENSG00000185122.6), *Hsf2* (ENSG00000025156.8) and *Hsf4* (ENSG00000102878.11) from donors between 21 and 70 years old (Genotype-Tissue Expression (GTEx) project (<https://gtexportal.org/home/> - dbGaP Accession [phs000424.v7.p2](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=phs000424.v7.p2))). Values are expressed in Transcripts Per Kilobase Million (TPM). For all tissues, number of samples is equal to 100 or higher, except for Substantia nigra (n = 88).

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Figure 2

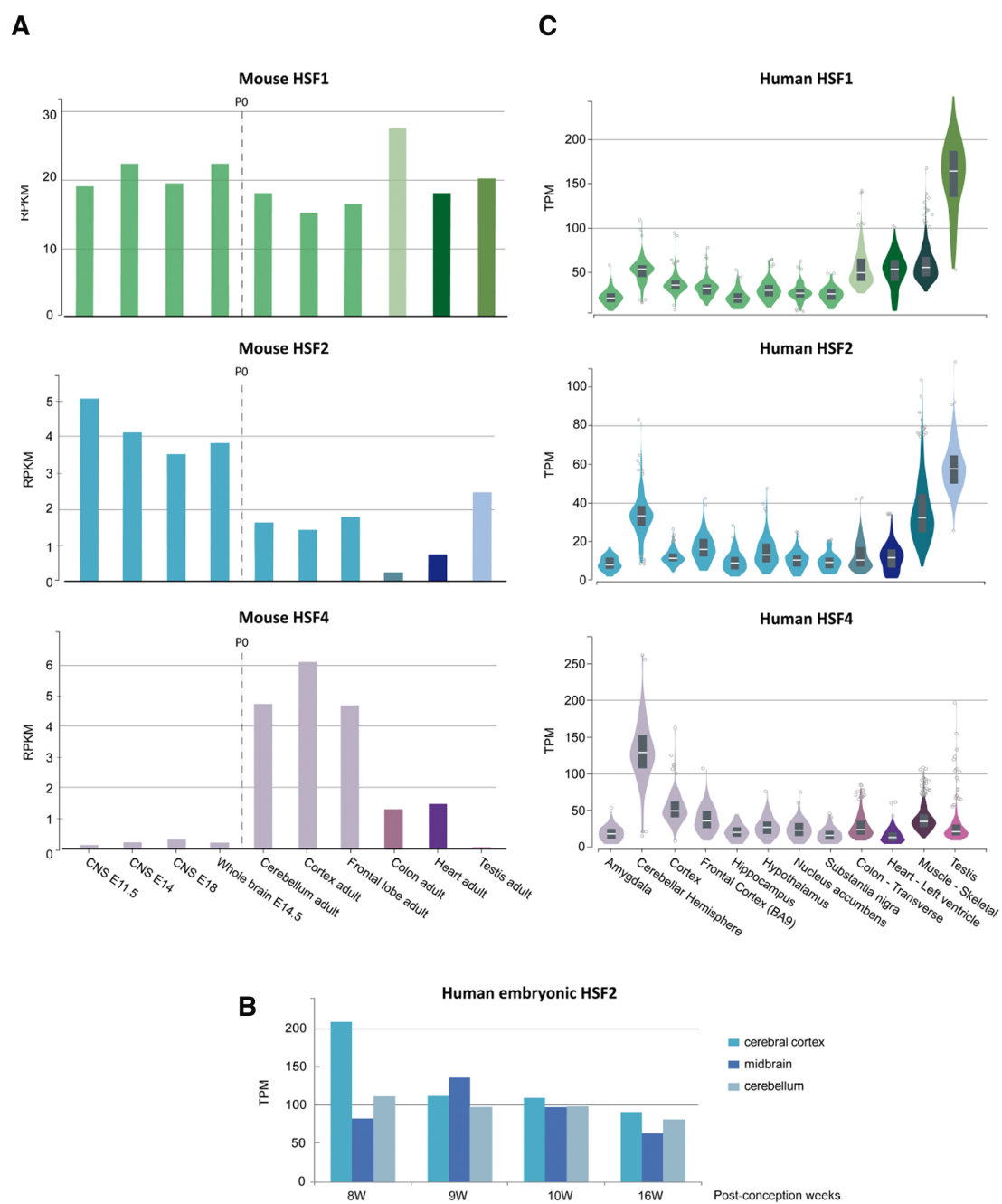


Figure 3. Dynamic expression of HSF2 during development.

(A) HSF2- β gal expression (in blue; knock-in of β -geo reporter at the *Hsf2* gene locus) was used as a reporter for *Hsf2* gene transcription in knock-in *Hsf2* embryos (as described, [77]). (a,b) Coronal sections (a) and parasagittal section (b) of embryos at day 11.5 (E11.5) of gestation. At E11.5, the *Hsf2* gene is enriched in the ventral forebrain region and expressed in midbrain and hindbrain regions. (c,d) Coronal sections of embryos at E15.5 and E17.5, respectively. At E15.5, *Hsf2* gene is expressed in the proliferative vz/svz, whereas at E17.5, it is also expressed in the cortical plate, and absent from the iz. Scale bars: (a,b) 1 mm; (c,d) 200 μ M.

(B) HSF2 expression pattern (green) in neural progenitors of the proliferative zone (vz; Tuj1 negative), in the dorsal telencephalon. Epifluorescence detection on coronal sections (as described, [78]) with an anti-HSF2 Antibody (3E2, Upstate); anti-beta III-tubulin (Tuj1; Sigma), a marker of young postmitotic neurons and DAPI to label nuclei. Scale bar, 50 μ M.

(C) HSF1 and HSF2 expression profiles at E16.5 in the cortical plate. Immunohistochemistry was performed as described [13] on coronal sections. Scale bar, 50 μ M.

(cp) cortical plate, (ge) ganglionic eminence, (iz) intermediate zone, (lv) luminal vesicle, (ob) olfactory bulb, (tv) telencephalic vesicle, (svz) subventricular zone.

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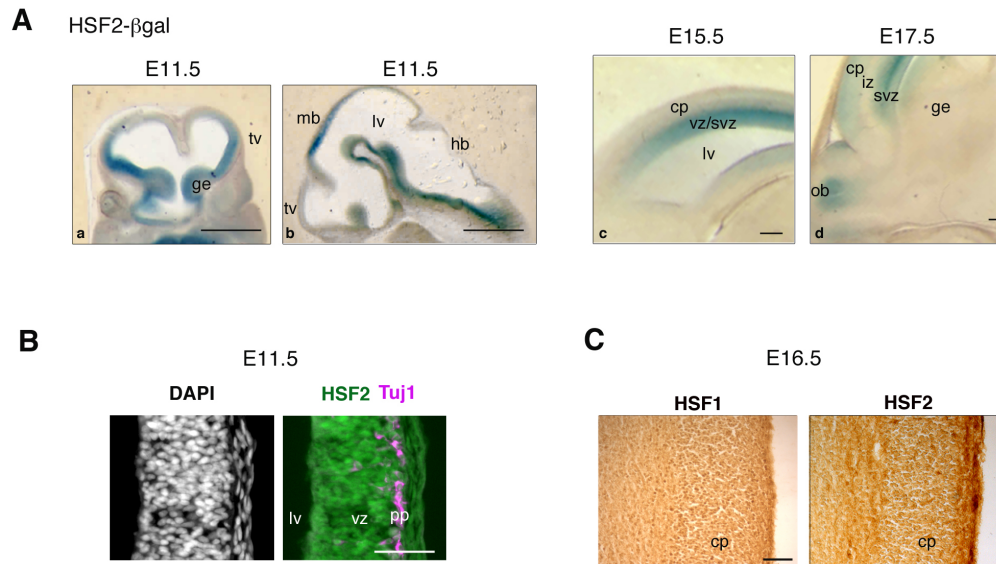
Figure 3

Figure 4. Schematized overview of the expression, subcellular localization, DNA-binding activity and oligomerization of HSF1 and HSF2, during rodent cortex development.

(A) Summary of relative protein expression of HSF1 (in green) and HSF2 (in blue) between embryonic days 9 or 11 (E9 or E11) and postnatal day 22 (P22), according to Western blot results from mice cortices [13,74,76,77], see also Fig. 4). The darker, the color, the highest the protein levels are (arbitrary scale). **Summary of the subcellular localization** data obtained by immunofluorescence, immuno-histochemistry, and Western blot experiments in mice [13,77,89] or rats [79,84,85]. The main or minor subcellular localization are indicated as +++ or +. **DNA-binding activity of HSF1 and HSF2** during cortex development was obtained by electromobility shift assay (EMSA) or ChIP-qPCR experiments [13,76,78,89], see also Fig. 6). In our hands, very low levels of HSF1 and HSF2 activities, if any, were detected between P0 and P4 in accordance with the protein levels (Fig. 4, [13], [VM, unpublished data]. Scale is arbitrary. **(B) Major homo- and hetero-oligomerization status of HSF1 and HSF2 in the pre- and postnatal brain.** Hypothetical oligomerization status of HSF1 (in green) and HSF2 (in blue) based on EMSA from cortical extracts [13,76-78], see also Fig. 6), chemical cross-linking followed by native electrophoresis, or pore exclusion limit electrophoresis experiments, in mouse fetal cortices, mouse neural cells and human cell lines [13,47,83], ChIP experiments in the prenatal cortex [13,89], and structural studies [28,31,32]. Before birth, HSF2 (in blue) is active in a homotrimer conformation, whereas HSF1 (in green) is present in an inactive form, likely as a monomer. Between P12 and P19, HSF1 and HSF2 bind to DNA (possibly) as heterotrimer (see also Fig. 6; homotrimers could also exist, but at a very low level, undetectable in EMSA). The stoichiometry of these heterotrimers in term of HSF1 and HSF2 molecules is not known (indicated by a question mark). From P19, active HSF1 forms homotrimers (see also Fig. 6). To our knowledge, there is no information on the oligomerization status of the inactive form of HSF2, in the mouse cortex at this stage (indicated by a question mark). It could thus exist as a monomer, similarly to HSF1, or as a homodimer, as detected in human K562 cells [47].

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Figure 4

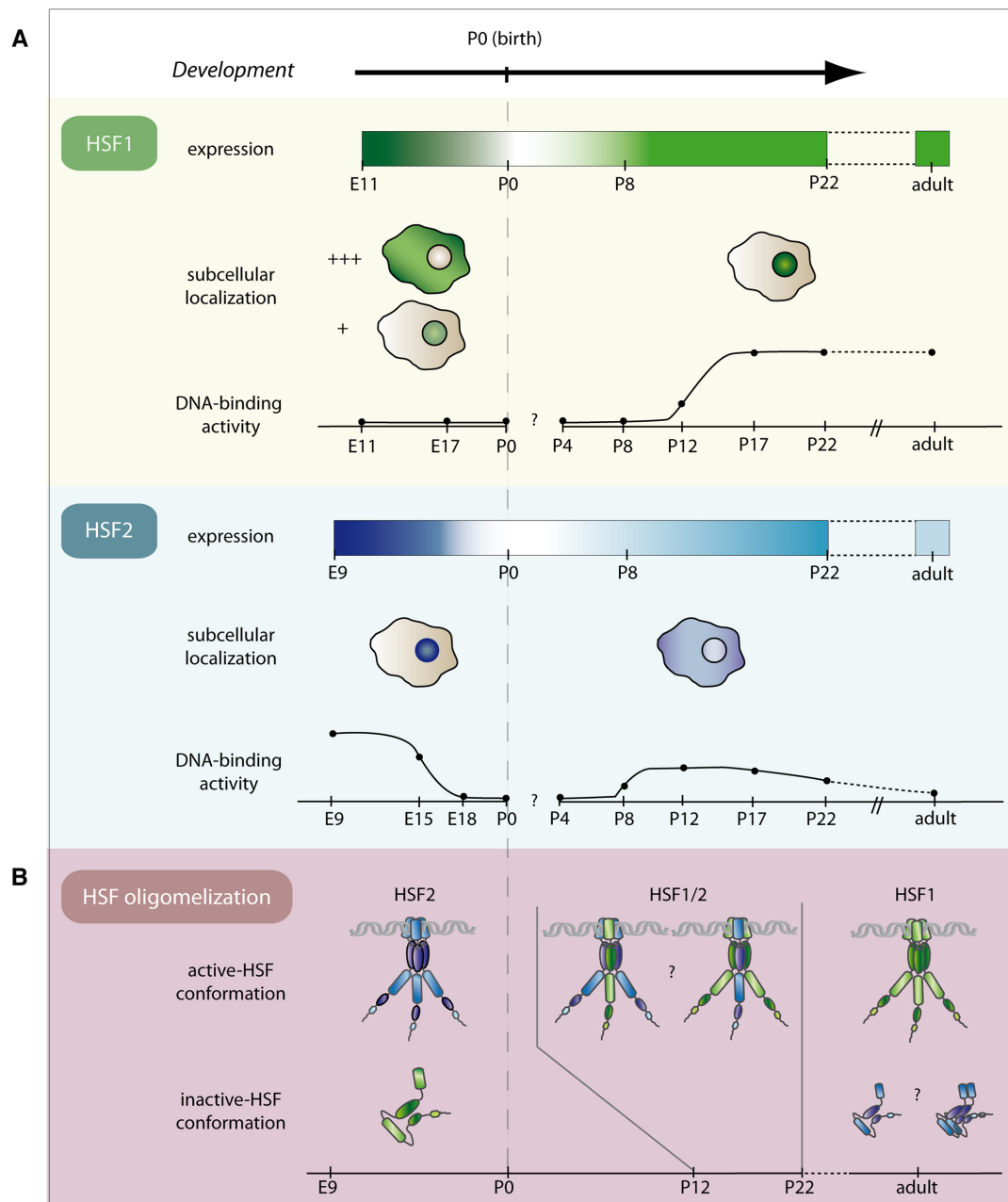


Figure 5. Expression levels of HSF1 and HSF2 proteins in mouse postnatal cortices. Cortex lysates obtained at indicated stages were subjected to 8% SDS-PAGE migration and immunoblotted with (A) rabbit anti-HSF1 antibody (sc-9144; Santa Cruz Biotechnologies) or (B) mouse anti-HSF2 antibody (sc-74529; Santa Cruz Biotechnologies) or (C) anti-HSF4 antibody (Gift from Prof. A ; Nakai). Actin serves as a loading control. Asteriks indicated possibly postranslationally modified HSF1 or HSF2 forms. Protein extraction and blotting was performed as described in Mezger *et al.*, 1989 [137], Rallu *et al.*, 1997 [76], and Fujimoto *et al.*, 2004 [138]. Dark asterisk points to a high molecular weight HSF2 form likely post-translationally modified. The and asterisk point to the HSF2 forms of the classical (red arrowhead), or higher molecular weights (red asterisks), possibly deriving from alternative splicing events, different transcription starts, or post-translational modifications [76]. KO: *Hsf2*^{-/-} cortical extracts; ns, non-specific bands.

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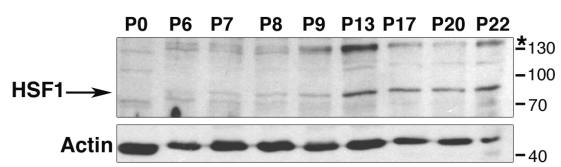
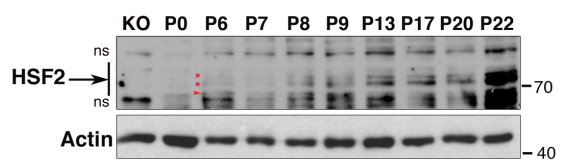
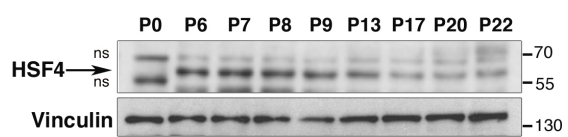
Figure 5**A****B****C**

Figure 6. HSF binding activities at early stages of chicken development.

Analysis of the presence of HSF-HSE complexes by electromobility shift assay (EMSA) at 32h of development, equivalent to stage 9 Hamburger and Hamilton (9HH) plus 30 min (32.5h; stage 9HH early), plus 2 hours (34h; stage 9HH late); plus 4 hours (36h; stage 10HH early), plus 6 hours (38h; stage 10HH late), plus 24 hours of development (56h; stage 16HH). A positive control for HSF3 DNA-binding activity is provided using embryos that were heat shocked for 1hr at 44°C (56h + HS). In normal developmental conditions, from 32.5h to 56h of development, no supershift was observed either with anti-HSF1 ($\alpha 1$) or anti-HSF3 sera ($\alpha 3$), in contrast to the HSF-HSE complexes supershift observed with anti-HSF2 antibodies ($\alpha 2$; lower intensity of the complex). The remaining vestigial but sharp band observed with anti-HSF2 is not a specific binding, since it cannot be displaced by non-radioactive HSE oligonucleotides, and is sometimes observed in EMSA [77]. Experimental conditions: commercial fertilized hens eggs were incubated in a humidified room at 38°C during 32 to 56 hours. Embryos were recovered in ice-cold PBS solution (*Invitrogen*) and tissues dissected. For EMSA experiments, protein extraction were performed as described in Mezger *et al.*, 1989 [137] and Rallu *et al.*, 1997 [76].

HSF-HSE: HSF-HSE retarded complexes. **CHBA:** constitutive HSE-binding activity. **ns:** non specific. **Free:** unbound oligonucleotides.

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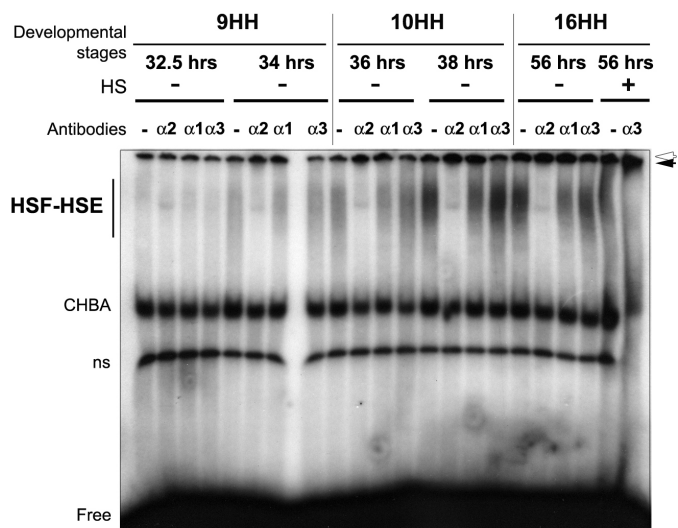
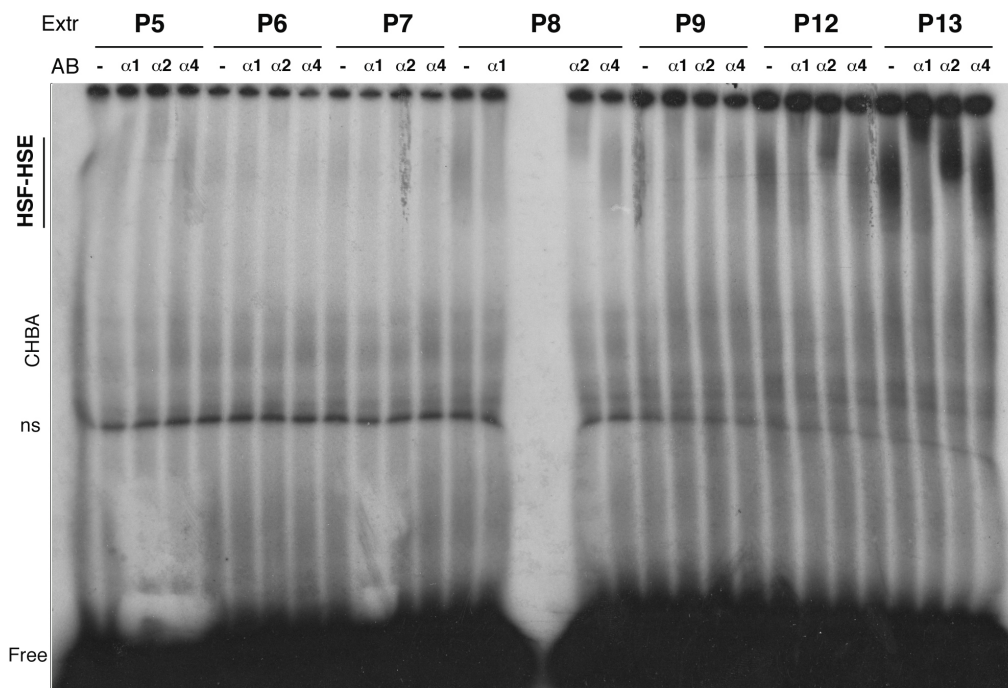
Figure 6

Figure 7. HSF binding activities in mouse cortical postnatal development.

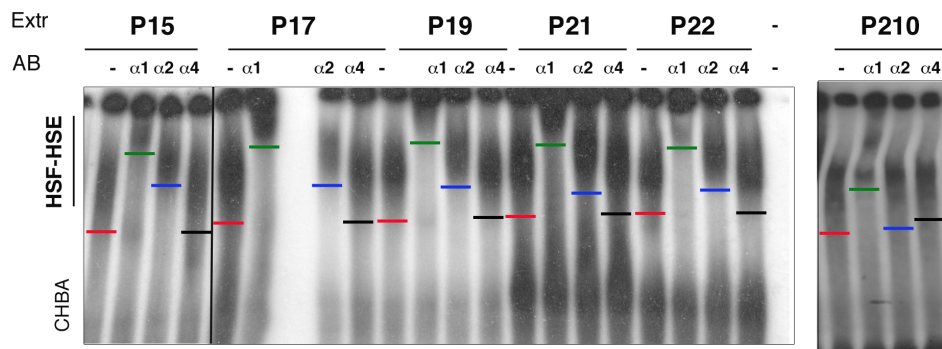
Analysis of the presence of HSF-HSE complexes by electromobility shift assay (EMSA) in the mouse cortex from postnatal day P5 to P13 (**A**), P13 to P22 (**B**), and P210 (7 month-old mouse; **B** and **C**) as in Rallu *et al.*, 1997 [76] and El Fatimy *et al.*, 2014 [13]. EMSA protocol and abbreviations, as in Fig. 5. Red bars underline the position of the HSF-HSE complex when no antibody has been added. The specific supershift induced by the addition of anti-HSF1 ($\alpha 1$) or anti-HSF2 antibodies ($\alpha 2$) is illustrated by green and blue bars, respectively. Anti-HSF4 antibodies ($\alpha 4$, [138]) do not supershift the HSF-HSE complex as underlined by black bars. The HSE probe can accept only one trimer [13]. Therefore, when only anti-HSF1 antibody is able to totally shift the HSF-HSE complex in EMSA (as at P210, in **B** and **C**), but not anti-HSF2 antibody, this indicates that the complex is composed of HSF1 only (likely as a HSF1 homotrimer, based on what is known about the oligomerization status of active HSF1, see also Fig. 3B). Same conclusions when supershift is observed only with anti-HSF2 antibodies (likely as a HSF2 homotrimer; as at E17.5, in **C**); [13], see also Fig. 3B). When either antibody can supershift the HSF-HSE, the HSF-HSE complex is composed of both HSF1 and HSF2, probably as heterotrimers ([13], see also Fig. 3B).

Figure 7

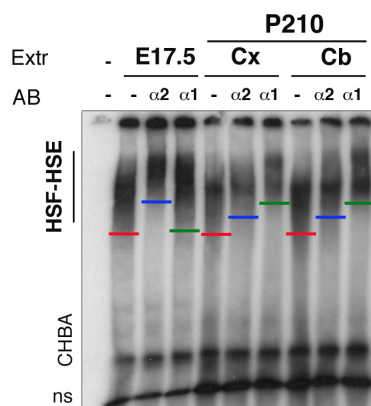
A



B



C



Acknowledgements

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References

- [1] C.S. Parker, J. Topol, A *Drosophila* RNA polymerase II transcription factor binds to the regulatory site of an hsp 70 gene, *Cell*. 37 (1984) 273–283.
- [2] C. Wu, Activating protein factor binds in vitro to upstream control sequences in heat shock gene chromatin, *Nature*. 311 (1984) 81–84. doi:10.1038/311081a0.
- [3] M. Åkerfelt, R.I. Morimoto, L. Sistonen, Heat shock factors: integrators of cell stress, development and lifespan, *Nat. Rev. Mol. Cell Biol.* 11 (2010) 545–555. doi:10.1038/nrm2938.
- [4] R. Abane, V. Mezger, Roles of heat shock factors in gametogenesis and development: Role of the HSF family in development, *FEBS J.* 277 (2010) 4150–4172. doi:10.1111/j.1742-4658.2010.07830.x.
- [5] R. Gomez-Pastor, E.T. Burchfiel, D.J. Thiele, Regulation of heat shock transcription factors and their roles in physiology and disease, *Nat. Rev. Mol. Cell Biol.* 19 (2018) 4–19. doi:10.1038/nrm.2017.73.
- [6] F. Miozzo, D. Sabéran-Djoneidi, V. Mezger, HSFs, Stress Sensors and Sculptors of Transcription Compartments and Epigenetic Landscapes, *J. Mol. Biol.* 427 (2015) 3793–3816. doi:10.1016/j.jmb.2015.10.007.
- [7] R. Gomez-Pastor, E.T. Burchfiel, D.W. Neef, A.M. Jaeger, E. Cabisco, S.U. McKinstry, A. Doss, A. Aballay, D.C. Lo, S.S. Akimov, C.A. Ross, C. Eroglu, D.J. Thiele, Abnormal degradation of the neuronal stress-protective transcription factor HSF1 in Huntington’s disease, *Nat. Commun.* 8 (2017) 14405. doi:10.1038/ncomms14405.
- [8] M. Fujimoto, A. Nakai, The heat shock factor family and adaptation to proteotoxic stress: Evolution and function of the HSF family, *FEBS J.* 277 (2010) 4112–4125. doi:10.1111/j.1742-4658.2010.07827.x.
- [9] R. Takii, M. Fujimoto, Y. Matsuura, F. Wu, N. Oshibe, E. Takaki, A. Katiyar, H. Akashi, T. Makino, M. Kawata, A. Nakai, HSF1 and HSF3 cooperatively regulate the heat shock response in lizards, *PLOS ONE*. 12 (2017) e0180776. doi:10.1371/journal.pone.0180776.
- [10] E. Takaki, M. Fujimoto, K. Sugahara, T. Nakahari, S. Yonemura, Y. Tanaka, N. Hayashida, S. Inouye, T. Takemoto, H. Yamashita, A. Nakai, Maintenance of olfactory neurogenesis requires HSF1, a major heat shock transcription factor in mice, *J. Biol. Chem.* 281 (2006) 4931–4937. doi:10.1074/jbc.M506911200.
- [11] M. Åkerfelt, A. Vihervaara, A. Laiho, A. Conter, E.S. Christians, L. Sistonen, E. Henriksson, Heat Shock Transcription Factor 1 Localizes to Sex Chromatin during Meiotic Repression, *J. Biol. Chem.* 285 (2010) 34469–34476. doi:10.1074/jbc.M110.157552.
- [12] T. Shinkawa, K. Tan, M. Fujimoto, N. Hayashida, K. Yamamoto, E. Takaki, R. Takii, R. Prakasam, S. Inouye, V. Mezger, others, Heat shock factor 2 is required for maintaining proteostasis against febrile-range thermal stress and polyglutamine aggregation, *Mol. Biol. Cell*. 22 (2011) 3571–3583. doi:10.1091/mbc.E11-04-0330.

- [13] R. El Fatimy, F. Miozzo, A. Le Mouel, R. Abane, L. Schwendimann, D. Saberan-Djoneidi, A. de Thonel, I. Massaoudi, L. Paslaru, K. Hashimoto-Torii, E. Christians, P. Rakic, P. Gressens, V. Mezger, Heat shock factor 2 is a stress-responsive mediator of neuronal migration defects in models of fetal alcohol syndrome, *EMBO Mol. Med.* 6 (2014) 1043–1061. doi:10.15252/emmm.201303311.
- [14] F. Miozzo, H. Arnoux, A. De Thonel, A.L. Schang, D. Saberan-Djoneidi, A. Baudry, B. Schneider, V. Mezger, Alcohol exposure promotes DNA methyltransferase DNMT3A up-regulation through reactive oxygen species-dependent mechanisms, *Cell Stress Chaperones.* (2018).
- [15] S. Lecomte, F. Desmots, F. Le Masson, P. Le Goff, D. Michel, E.S. Christians, Y. Le Dréan, Roles of heat shock factor 1 and 2 in response to proteasome inhibition: consequence on p53 stability, *Oncogene.* 29 (2010) 4216–4224. doi:10.1038/onc.2010.171.
- [16] A. Rossi, A. Riccio, M. Coccia, E. Trotta, S. La Frazia, M.G. Santoro, The proteasome inhibitor bortezomib is a potent inducer of zinc finger an1-type domain 2a gene expression: role of heat shock factor 1 (HSF1)-heat shock factor 2 (HSF2) heterocomplexes, *J. Biol. Chem.* 289 (2014) 12705–12715. doi:10.1074/jbc.M113.513242.
- [17] J. Joutsen, A.J. Da Silva, J.C. Luoto, M.A. Budzynski, A.S. Nylund, A. de Thonel, J.P. Concordet, V. Mezger, D. Sabéran-Djoneidi, E. Henriksson, L. Sistonen. Heat Shock Factor 2 Protects against Proteotoxicity by Maintaining Cell-Cell Adhesion. *Cell Rep.* 30 (2020) 583-597.e6. doi: 10.1016/j.celrep.2019.12.037.
- [18] M. Tanabe, A. Nakai, Y. Kawazoe, K. Nagata, Different thresholds in the responses of two heat shock transcription factors, HSF1 and HSF3, *J. Biol. Chem.* 272 (1997) 15389–15395.
- [19] M. Tanabe, Y. Kawazoe, S. Takeda, R.I. Morimoto, K. Nagata, A. Nakai, Disruption of the HSF3 gene results in the severe reduction of heat shock gene expression and loss of thermotolerance, *EMBO J.* 17 (1998) 1750–1758. doi:10.1093/emboj/17.6.1750.
- [20] Y. Kawazoe, M. Tanabe, N. Sasai, K. Nagata, A. Nakai, HSF3 is a major heat shock responsive factor during chicken embryonic development, *Eur. J. Biochem.* 265 (1999) 688–697.
- [21] A. Nakai, R.I. Morimoto, Characterization of a novel chicken heat shock transcription factor, heat shock factor 3, suggests a new regulatory pathway, *Mol. Cell. Biol.* 13 (1993) 1983–1997.
- [22] M. Fujimoto, S. Inouye, A. Nakai, Physiological roles of heat shock transcription factors, *Seikagaku.* 76 (2004) 419–428.
- [23] J.-N. Min, Y. Zhang, D. Moskophidis, N.F. Mivechi, Unique contribution of heat shock transcription factor 4 in ocular lens development and fiber cell differentiation, *Genesis.* 40 (2004) 205–217. doi:10.1002/gene.20087.
- [24] L. Bu, Y. Jin, Y. Shi, R. Chu, A. Ban, H. Eiberg, L. Andres, H. Jiang, G. Zheng, M. Qian, B. Cui, Y. Xia, J. Liu, L. Hu, G. Zhao, M.R. Hayden, X. Kong, Mutant DNA-binding domain of HSF4 is associated with autosomal dominant lamellar and Marner cataract, *Nat. Genet.* 31 (2002) 276–278. doi:10.1038/ng921.
- [25] P.K Sorger, H.C.Nelson, Trimerization of a yeast transcriptional activator via a coiled-coil motif. *Cell* 59 (1989) 807–813. DOI: 10.1016/0092-8674(89)90604-1
- [26] S.K. Rabindran, R.I. Haroun, J. Clos, J. Wisniewski, C. Wu, Regulation of heat shock factor trimer formation: role of a conserved leucine zipper, *Science.* 259 (1993) 230–234. doi:10.1126/science.8421783.
- [27] J. Zuo, R. Baler, Dahl, G., R. Voellmy. Activation of the DNA-binding ability of human heat shock transcription factor 1 may involve the transition from an intramolecular to an intermolecular triple-stranded coiled-coil structure. *Mol. Cell. Biol.* 14 (1994) 7557–7568. DOI: 10.1128/mcb.14.11.7557
- [28] C. Wu, Heat shock transcription factors: structure and regulation, *Annu. Rev. Cell Dev. Biol.* 11 (1995) 441–469. doi:10.1146/annurev.cb.11.110195.002301.
- [29] Farkas, T., Kutsikova, Y.A. & Zimarino, V. Intramolecular repression of mouse heat shock factor 1. *Mol. Cell. Biol.* 18 (1998) 906–918. DOI: 10.1128/mcb.18.2.906

- [30] O. Littlefield, H.C. Nelson, H.C. A new use for the 'wing' of the 'winged' helix-turn-helix motif in the HSF-DNA cocrystal. *Nat. Struct. Biol.* 6 (1999) 464–470. DOI: 10.1038/8269
- [31] A.M. Jaeger, C.W. Pemble, L. Sistonen, D.J. Thiele, Structures of HSF2 reveal mechanisms for differential regulation of human heat-shock factors, *Nat. Struct. Mol. Biol.* 23 (2016) 147–154. doi:10.1038/nsmb.3150.
- [32] T. Neudegger, J. Verghese, M. Hayer-Hartl, F.U. Hartl, A. Bracher, Structure of human heat-shock transcription factor 1 in complex with DNA, *Nat. Struct. Mol. Biol.* 23 (2016) 140–146. doi:10.1038/nsmb.3149.
- [33] P.E. Kroeger, R.I. Morimoto, Selection of new HSF1 and HSF2 DNA-binding sites reveals difference in trimer cooperativity, *Mol. Cell. Biol.* 14 (1994) 7592–7603.
- [34] M. Manuel, M. Rallu, M.T. Loones, V. Zimarino, V. Mezger, M. Morange. Determination of the consensus binding sequence for the purified embryonic heat shock factor 2. *Eur J Biochem.* 269 (2002) 2527–37. DOI: 10.1046/j.1432-1033.2002.02917.x
- [35] L. Pirkkala, P. Nykänen, L. Sistonen, Roles of the heat shock transcription factors in regulation of the heat shock response and beyond, *FASEB J.* 15 (2001) 1118–1131.
- [36] N.D. Trinklein, J.I. Murray, S.J. Hartman, D. Botstein, R.M. Myers, The role of heat shock transcription factor 1 in the genome-wide regulation of the mammalian heat shock response, *Mol. Biol. Cell.* 15 (2004) 1254–1261. doi:10.1091/mbc.E03-10-0738.
- [37] A. Vihervaara, C. Sergelius, J. Vasara, M.A. Blom, A.N. Elsing, P. Roos-Mattjus, L. Sistonen, Transcriptional response to stress in the dynamic chromatin environment of cycling and mitotic cells, *Proc. Natl. Acad. Sci.* 110 (2013) E3388–E3397. doi:10.1073/pnas.1305275110.
- [38] M.L. Mendillo, S. Santagata, M. Koeva, G.W. Bell, R. Hu, R.M. Tamimi, E. Fraenkel, T.A. Ince, L. Whitesell, S. Lindquist, HSF1 Drives a Transcriptional Program Distinct from Heat Shock to Support Highly Malignant Human Cancers, *Cell.* 150 (2012) 549–562. doi:10.1016/j.cell.2012.06.031.
- [39] P. Ostling, J.K. Bjork, P. Roos-Mattjus, V. Mezger, L. Sistonen, Heat Shock Factor 2 (HSF2) Contributes to Inducible Expression of hsp Genes through Interplay with HSF1, *J. Biol. Chem.* 282 (2007) 7077–7086. doi:10.1074/jbc.M607556200.
- [40] A. Sandqvist, J.K. Björk, M. Åkerfelt, Z. Chitikova, A. Grichine, C. Vourc'h, C. Jolly, T.A. Salminen, Y. Nymalm, L. Sistonen, Heterotrimerization of Heat-Shock Factors 1 and 2 Provides a Transcriptional Switch in Response to Distinct Stimuli, *Mol. Biol. Cell.* 20 (2009) 1340–1347. doi:10.1091/mbc.E08-08-0864.
- [41] Y. Shi, P.E. Kroeger, R.I. Morimoto, The carboxyl-terminal transactivation domain of heat shock factor 1 is negatively regulated and stress responsive, *Mol. Cell. Biol.* 15 (1995) 4309–4318. doi:10.1128/mcb.15.8.4309.
- [42] J. Zuo, D. Rungger, R. Voellmy, Multiple layers of regulation of human heat shock transcription factor 1., *Mol. Cell. Biol.* 15 (1995) 4319–4330.
- [43] E.K. Sullivan, C.S. Weirich, J.R. Guyon, S. Sif, R.E. Kingston, Transcriptional Activation Domains of Human Heat Shock Factor 1 Recruit Human SWI/SNF, *Mol. Cell. Biol.* 21 (2001) 5826–5837. doi:10.1128/MCB.21.17.5826-5837.2001.
- [44] J. Joutsen, L. Sistonen, Tailoring of Proteostasis Networks with Heat Shock Factors, *Cold Spring Harb. Perspect. Biol.* 11 (2019) a034066. doi:10.1101/cshperspect.a034066.
- [45] J.O. Hensold, C.R. Hunt, S.K. Calderwood, D.E. Housman, R.E. Kingston, DNA binding of heat shock factor to the heat shock element is insufficient for transcriptional activation in murine erythroleukemia cells., *Mol. Cell. Biol.* 10 (1990) 1600–1608. doi:10.1128/MCB.10.4.1600.
- [46] N. Hentze, L. Le Breton, J. Wiesner, G. Kempf, M.P. Mayer, Molecular mechanism of thermosensory function of human heat shock transcription factor Hsf1, *ELife.* 5 (2016) e11576. doi:10.7554/eLife.11576.
- [47] L. Sistonen, K.D. Sarge, R.I. Morimoto, Human heat shock factors 1 and 2 are differentially activated and can synergistically induce hsp70 gene transcription, *Mol. Cell. Biol.* 14 (1994) 2087–2099.

- [48] L.A. Sheldon, R.E. Kingston, Hydrophobic coiled-coil domains regulate the subcellular localization of human heat shock factor 2, *Genes Dev.* 7 (1993) 1549–1558.
- [49] M. Vujanac, A. Fenaroli, V. Zimarino, Constitutive nuclear import and stress-regulated nucleocytoplasmic shuttling of mammalian heat-shock factor 1, *Traffic.* 6 (2005) 214–229. doi:10.1111/j.1600-0854.2005.00266.x.
- [50] A. Nakai, T. Ishikawa, A nuclear localization signal is essential for stress-induced dimer-to-trimer transition of heat shock transcription factor 3. *J Biol Chem.* 275 (2000) 34665–34671.
- [51] T. Somasundaram, S.P. Bhat, Canonical heat shock element in the alpha B-crystallin gene shows tissue-specific and developmentally controlled interactions with heat shock factor, *J. Biol. Chem.* 275 (2000) 17154–17159. doi:10.1074/jbc.M000304200.
- [52] J. Li, L. Chauve, G. Phelps, R.M. Briemann, R.I. Morimoto, E2F coregulates an essential HSF developmental program that is distinct from the heat-shock response, *Genes Dev.* 30 (2016) 2062–2075. doi:10.1101/gad.283317.116.
- [53] H. Xiao, O. Perisic, J.T. Lis, Cooperative binding of Drosophila heat shock factor to arrays of a conserved 5 bp unit, *Cell.* 64 (1991) 585–593.
- [54] R.S. Hilgarth, Y. Hong, O.-K. Park-Sarge, K.D. Sarge, Insights into the regulation of heat shock transcription factor 1 SUMO-1 modification, *Biochem. Biophys. Res. Commun.* 303 (2003) 196–200. doi:10.1016/S0006-291X(03)00312-7.
- [55] T. Guettouche, F. Boellmann, W.S. Lane, R. Voellmy, Analysis of phosphorylation of human heat shock factor 1 in cells experiencing a stress, *BMC Biochem.* 6 (2005) 4.
- [56] S. Raychaudhuri, C. Loew, R. Körner, S. Pinkert, M. Theis, M. Hayer-Hartl, F. Buchholz, F.U. Hartl, Interplay of acetyltransferase EP300 and the proteasome system in regulating heat shock transcription factor 1, *Cell.* 156 (2014) 975–985. doi:10.1016/j.cell.2014.01.055.
- [57] N. Kourtis, R.S. Moubarak, B. Aranda-Orgilles, K. Lui, I.T. Aydin, T. Trimarchi, F. Darvishian, C. Salvaggio, J. Zhong, K. Bhatt, E.I. Chen, J.T. Celebi, C. Lazaris, A. Tsirigos, I. Osman, E. Hernando, I. Aifantis, FBXW7 modulates cellular stress response and metastatic potential through HSF1 post-translational modification, *Nat. Cell Biol.* 17 (2015) 322–332. doi:10.1038/ncb3121.
- [58] Z. Tang, S. Dai, Y. He, R.A. Doty, L.D. Shultz, S.B. Sampson, C. Dai, MEK Guards Proteome Stability and Inhibits Tumor-Suppressive Amyloidogenesis via HSF1, *Cell.* 160 (2015) 729–744. doi:10.1016/j.cell.2015.01.028.
- [59] N. Hashikawa, N. Yamamoto, H. Sakurai, Different Mechanisms Are Involved in the Transcriptional Activation by Yeast Heat Shock Transcription Factor through Two Different Types of Heat Shock Elements, *J. Biol. Chem.* 282 (2007) 10333–10340. doi:10.1074/jbc.M609708200.
- [60] M.A. Budzyński, M.C. Puustinen, J. Joutsen, L. Sistonen, Uncoupling Stress-Inducible Phosphorylation of Heat Shock Factor 1 from Its Activation, *Mol. Cell. Biol.* 35 (2015) 2530–2540. doi:10.1128/MCB.00816-14.
- [61] A. Murshid, S.-D. Chou, T. Prince, Y. Zhang, A. Bharti, S.K. Calderwood, Protein kinase A binds and activates heat shock factor 1, *PloS One.* 5 (2010) e13830. doi:10.1371/journal.pone.0013830.
- [62] Y. Zhang, A. Murshid, T. Prince, S.K. Calderwood, Protein kinase A regulates molecular chaperone transcription and protein aggregation, *PloS One.* 6 (2011) e28950. doi:10.1371/journal.pone.0028950.
- [63] S.D. Westerheide, J. Anckar, S.M. Stevens, L. Sistonen, R.I. Morimoto, Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1, *Science.* 323 (2009) 1063–1066. doi:10.1126/science.1165946.
- [64] R. Raynes, K.M. Pombier, K. Nguyen, J. Brunquell, J.E. Mendez, S.D. Westerheide, The SIRT1 modulators AROS and DBC1 regulate HSF1 activity and the heat shock response, *PloS One.* 8 (2013) e54364. doi:10.1371/journal.pone.0054364.
- [65] E. Zelin, B.C. Freeman, Lysine deacetylases regulate the heat shock response including the age-associated impairment of HSF1, *J. Mol. Biol.* 427 (2015) 1644–1654. doi:10.1016/j.jmb.2015.02.010.

- [66] V. Hietakangas, J. Anckar, H.A. Blomster, M. Fujimoto, J.J. Palvimo, A. Nakai, L. Sistonen, PDSM, a motif for phosphorylation-dependent SUMO modification, *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 45–50. doi:10.1073/pnas.0503698102.
- [67] J. Anckar, L. Sistonen, Heat shock factor 1 as a coordinator of stress and developmental pathways, in: *Mol. Asp. Stress Response Chaperones Membr. Netw.*, Springer, 2007: pp. 78–88. http://link.springer.com/chapter/10.1007/978-0-387-39975-1_8 (accessed March 25, 2017).
- [68] J. Anckar, V. Hietakangas, K. Denessiouk, D.J. Thiele, M.S. Johnson, L. Sistonen, Inhibition of DNA Binding by Differential Sumoylation of Heat Shock Factors, *Mol. Cell. Biol.* 26 (2006) 955–964. doi:10.1128/MCB.26.3.955-964.2006.
- [69] Y. Tateishi, M. Ariyoshi, R. Igarashi, H. Hara, K. Mizuguchi, A. Seto, A. Nakai, T. Kokubo, H. Tochio, M. Shirakawa, Molecular Basis for SUMOylation-dependent Regulation of DNA Binding Activity of Heat Shock Factor 2, *J. Biol. Chem.* 284 (2008) 2435–2447. doi:10.1074/jbc.M806392200.
- [70] Kawazoe Y, Nakai A, Tanabe M, Nagata K. Proteasome inhibition leads to the activation of all members of the heat-shock-factor family. *Eur J Biochem.* 1998 Jul 15;255(2):356-62. DOI: 10.1046/j.1432-1327.1998.2550356.x
- [71] A. Mathew, S.K. Mathur, R.I. Morimoto, Heat shock response and protein degradation: regulation of HSF2 by the ubiquitin-proteasome pathway, *Mol. Cell. Biol.* 18 (1998) 5091–5098. DOI: 10.1128/mcb.18.9.5091
- [72] A.N. Elsing, C. Aspelin, J.K. Björk, H.A. Bergman, S.V. Himanen, M.J. Kallio, P. Roos-Mattjus, L. Sistonen, Expression of HSF2 decreases in mitosis to enable stress-inducible transcription and cell survival, *J. Cell Biol.* 206 (2014) 735–749. doi:10.1083/jcb.201402002.
- [73] J.K. Ahlskog, J.K. Björk, A.N. Elsing, C. Aspelin, M. Kallio, P. Roos-Mattjus, L. Sistonen, Anaphase-promoting complex/cyclosome participates in the acute response to protein-damaging stress, *Mol. Cell. Biol.* 30 (2010) 5608–5620. doi:10.1128/MCB.01506-09.
- [74] A. de Thonel, J.K. Ahlskog, R. Abane, G. Pires, V. Dubreuil, J. Bertelet, A.L. Aalto, S. Naceri, M. Cordonnier, C. Benasolo, M. Sanial, A. Duchateau, A. Vihervaara, M.C. Puustinen, F. Miozzo, M. Henry, D. Bouvier, J.-P. Concordet, P. Fergelot, E. Lebigot, A. Verloes, P. Gressens, D. Lacombe, J. Gobbo, C. Garrido, S.D. Westerheide, M. Petitjean, O. Taboureau, F. Rodrigues-Lima, M. Lancaster, S. Passemart, D.S. Dioneidi, L. Sistonen, V. Mezger, CBP/EP300-dependent acetylation and stabilization of HSF2 are compromised in the rare disorder, Rubinstein-Taybi syndrome, *BioRxiv.* (2018) 481457. doi:10.1101/481457.
- [75] F.-L. Yeh, L.-Y. Hsu, B.-A. Lin, C.-F. Chen, I.-C. Li, S.-H. Tsai, T. Hsu, Cloning of zebrafish (*Danio rerio*) heat shock factor 2 (HSF2) and similar patterns of HSF2 and HSF1 mRNA expression in brain tissues, *Biochimie.* 88 (2006) 1983–1988. doi:10.1016/j.biochi.2006.07.005.
- [76] M. Rallu, M.T. Loones, Y. Lallemand, R. Morimoto, M. Morange, V. Mezger, Function and regulation of heat shock factor 2 during mouse embryogenesis, *Proc. Natl. Acad. Sci.* 94 (1997) 2392–2397.
- [77] M. Kallio, Y. Chang, M. Manuel, T.-P. Alastalo, M. Rallu, Y. Gitton, L. Pirkkala, M.-T. Loones, L. Paslaru, S. Larney, S. Hiard, M. Morange, L. Sistonen, V. Mezger, Brain abnormalities, defective meiotic chromosome synapsis and female subfertility in HSF2 null mice, *EMBO J.* 21 (2002) 2591–2601. doi:10.1093/emboj/21.11.2591.
- [78] Y. Chang, P. Östling, M. Aakerfelt, D. Trouillet, M. Rallu, Y. Gitton, R. El Fatimy, V. Fardeau, S. Le Crom, M. Morange, others, Role of heat-shock factor 2 in cerebral cortex formation and as a regulator of p35 expression, *Genes Dev.* 20 (2006) 836–847.
- [79] D. Walsh, Z. Li, Y. Wu, K. Nagata, Heat shock and the role of the HSPs during neural plate induction in early mammalian CNS and brain development, *Cell. Mol. Life Sci.* 53 (1997) 198–211.
- [80] M. Manuel, J. Sage, M.-G. Mattéi, M. Morange, V. Mezger, Genomic structure and chromosomal localization of the mouse *Hsf2* gene and promoter sequences, *Gene.* 232 (1999) 115–124.

- [81] P. Nykänen, T.-P. Alastalo, J. Ahlskog, N. Horelli-Kuitunen, L. Pirkkala, L. Sistonen, Genomic organization and promoter analysis of the human heat shock factor 2 gene, *Cell Stress Chaperones*. 6 (2001) 377–385.
- [82] S.-S. Lee, S.-H. Kwon, J.-S. Sung, M.-Y. Han, Y.-M. Park, Cloning and characterization of the rat Hsf2 promoter: a critical role of proximal E-box element and USF protein in Hsf2 regulation in different compartments of the brain, *Biochim. Biophys. Acta*. 1625 (2003) 52–63.
- [83] K.D. Sarge, S.P. Murphy, R.I. Morimoto, Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress, *Mol. Cell. Biol.* 13 (1993) 1392–1407.
- [84] I.R. Brown, S.J. Rush, Cellular localization of the heat shock transcription factors HSF1 and HSF2 in the rat brain during postnatal development and following hyperthermia, *Brain Res.* 821 (1999) 333–340.
- [85] A.J. Morrison, S.J. Rush, I.R. Brown, Heat shock transcription factors and the hsp70 induction response in brain and kidney of the hyperthermic rat during postnatal development, *J. Neurochem.* 75 (2000) 363–372.
- [86] M.A. Lancaster, M. Renner, C.-A. Martin, D. Wenzel, L.S. Bicknell, M.E. Hurles, T. Homfray, J.M. Penninger, A.P. Jackson, J.A. Knoblich, Cerebral organoids model human brain development and microcephaly, *Nature*. 501 (2013) 373–379. doi:10.1038/nature12517.
- [87] S. Velasco, A.J. Kedaigle, S.K. Simmons, A. Nash, M. Rocha, G. Quadrato, B. Paulsen, L. Nguyen, X. Adiconis, A. Regev, J.Z. Levin, P. Arlotta, Individual brain organoids reproducibly form cell diversity of the human cerebral cortex, *Nature*. (2019). doi:10.1038/s41586-019-1289-x.
- [88] J.G. Camp, F. Badsha, M. Florio, S. Kanton, T. Gerber, M. Wilsch-Bräuninger, E. Lewitus, A. Sykes, W. Hevers, M. Lancaster, J.A. Knoblich, R. Lachmann, S. Pääbo, W.B. Huttner, B. Treutlein, Human cerebral organoids recapitulate gene expression programs of fetal neocortex development, *Proc. Natl. Acad. Sci. U. S. A.* 112 (2015) 15672–15677. doi:10.1073/pnas.1520760112.
- [89] K. Hashimoto-Torii, M. Torii, M. Fujimoto, A. Nakai, R. El Fatimy, V. Mezger, M.J. Ju, S. Ishii, S. Chao, K.J. Brennand, F.H. Gage, P. Rakic, Roles of Heat Shock Factor 1 in Neuronal Response to Fetal Environmental Risks and Its Relevance to Brain Disorders. *Neuron*. 82 (2014) 560–572. doi:10.1016/j.neuron.2014.03.002.
- [90] Y. Hu, N.F. Mivechi, Association and Regulation of Heat Shock Transcription Factor 4b with both Extracellular Signal-Regulated Kinase Mitogen-Activated Protein Kinase and Dual-Specificity Tyrosine Phosphatase DUSP26, *Mol. Cell. Biol.* 26 (2006) 3282–3294. doi:10.1128/MCB.26.8.3282-3294.2006.
- [91] S. Uchida, K. Hara, A. Kobayashi, M. Fujimoto, K. Otsuki, H. Yamagata, T. Hobara, N. Abe, F. Higuchi, T. Shibata, S. Hasegawa, S. Kida, A. Nakai, Y. Watanabe, Impaired hippocampal spinogenesis and neurogenesis and altered affective behavior in mice lacking heat shock factor 1, *Proc. Natl. Acad. Sci.* 108 (2011) 1681–1686. doi:10.1073/pnas.1016424108.
- [92] M. Tanabe, N. Sasai, K. Nagata, X.D. Liu, P.C. Liu, D.J. Thiele, A. Nakai, The mammalian HSF4 gene generates both an activator and a repressor of heat shock genes by alternative splicing, *J. Biol. Chem.* 274 (1999) 27845–27856.
- [93] K. Hashimoto-Torii, Y.I. Kawasawa, A. Kuhn, P. Rakic, Combined transcriptome analysis of fetal human and mouse cerebral cortex exposed to alcohol, *Proc. Natl. Acad. Sci.* 108 (2011) 4212–4217. doi:10.1073/pnas.1100903108.
- [94] G. Wang, J. Zhang, D. Moskophidis, N.F. Mivechi, Targeted disruption of the heat shock transcription factor (hsf)-2 gene results in increased embryonic lethality, neuronal defects, and reduced spermatogenesis, *Genesis*. 36 (2003) 48–61. doi:10.1002/gene.10200.
- [95] D.R. McMillan, E. Christians, M. Forster, X. Xiao, P. Connell, J.-C. Plumier, X. Zuo, J. Richardson, S. Morgan, I.J. Benjamin, Heat Shock Transcription Factor 2 Is Not Essential for Embryonic Development, Fertility, or Adult Cognitive and Psychomotor Function in Mice, *Mol. Cell. Biol.* 22 (2002) 8005–8014. doi:10.1128/MCB.22.22.8005-8014.2002.

- [96] R. Ayala, T. Shu, L.-H. Tsai, Trekking across the brain: the journey of neuronal migration, *Cell*. 128 (2007) 29–43. doi:10.1016/j.cell.2006.12.021.
- [97] T. Sapir, M. Frotscher, T. Levy, E.-M. Mandelkow, O. Reiner, Tau's role in the developing brain: implications for intellectual disability, *Hum. Mol. Genet.* 21 (2012) 1681–1692. doi:10.1093/hmg/ddr603.
- [98] X.-D. Ju, Y. Guo, N.-N. Wang, Y. Huang, M.-M. Lai, Y.-H. Zhai, Y.-G. Guo, J.-H. Zhang, R.-J. Cao, H.-L. Yu, L. Cui, Y.-T. Li, X.-Z. Wang, Y.-Q. Ding, X.-J. Zhu, Both Myosin-10 isoforms are required for radial neuronal migration in the developing cerebral cortex, *Cereb. Cortex N. Y. N* 1991. 24 (2014) 1259–1268. doi:10.1093/cercor/bhs407.
- [99] J. Ko, S. Humbert, R.T. Bronson, S. Takahashi, A.B. Kulkarni, E. Li, L.H. Tsai, p35 and p39 are essential for cyclin-dependent kinase 5 function during neurodevelopment, *J. Neurosci. Off. J. Soc. Neurosci.* 21 (2001) 6758–6771.
- [100] Y. Tanaka, M. Kameoka, A. Itaya, K. Ota, K. Yoshihara, Regulation of HSF1-responsive gene expression by N-terminal truncated form of p73 α , *Biochem. Biophys. Res. Commun.* 317 (2004) 865–872. doi:10.1016/j.bbrc.2004.03.124.
- [101] N.E. Faulkner, D.L. Dujardin, C.Y. Tai, K.T. Vaughan, C.B. O'Connell, Y. Wang, R.B. Vallee, A role for the lissencephaly gene LIS1 in mitosis and cytoplasmic dynein function, *Nat. Cell Biol.* 2 (2000) 784–791. doi:10.1038/35041020.
- [102] Y. Feng, C.A. Walsh, Mitotic spindle regulation by Nde1 controls cerebral cortical size, *Neuron*. 44 (2004) 279–293.
- [103] J. Yingling, Y.H. Youn, D. Darling, K. Toyo-Oka, T. Pramparo, S. Hirotsune, A. Wynshaw-Boris, Neuroepithelial stem cell proliferation requires LIS1 for precise spindle orientation and symmetric division, *Cell*. 132 (2008) 474–486. doi:10.1016/j.cell.2008.01.026.
- [104] G.P. Demyanenko, M. Schachner, E. Anton, R. Schmid, G. Feng, J. Sanes, P.F. Maness, Close homolog of L1 modulates area-specific neuronal positioning and dendrite orientation in the cerebral cortex, *Neuron*. 44 (2004) 423–437. doi:10.1016/j.neuron.2004.10.016.
- [105] Y. Matsunaga, M. Noda, H. Murakawa, K. Hayashi, A. Nagasaka, S. Inoue, T. Miyata, T. Miura, K.-I. Kubo, K. Nakajima, Reelin transiently promotes N-cadherin-dependent neuronal adhesion during mouse cortical development, *Proc. Natl. Acad. Sci. U. S. A.* 114 (2017) 2048–2053. doi:10.1073/pnas.1615215114.
- [106] S. Inoue, K. Hayashi, K. Fujita, K. Tagawa, H. Okazawa, K.-I. Kubo, K. Nakajima, Drebrin-like (Dbrn1) Controls Neuronal Migration via Regulating N-Cadherin Expression in the Developing Cerebral Cortex, *J. Neurosci. Off. J. Soc. Neurosci.* 39 (2019) 678–691. doi:10.1523/JNEUROSCI.1634-18.2018.
- [107] H. Jinnou, M. Sawada, K. Kawase, N. Kaneko, V. Herranz-Pérez, T. Miyamoto, T. Kawae, T. Miyata, Y. Tabata, T. Akaike, J.M. García-Verdugo, I. Ajioka, S. Saitoh, K. Sawamoto, Radial Glial Fibers Promote Neuronal Migration and Functional Recovery after Neonatal Brain Injury, *Cell Stem Cell*. 22 (2018) 128–137.e9. doi:10.1016/j.stem.2017.11.005.
- [108] M.J. Molumby, R.M. Anderson, D.J. Newbold, N.K. Koblesky, A.M. Garrett, D. Schreiner, J.J. Radley, J.A. Weiner, γ -Protocadherins Interact with Neuroligin-1 and Negatively Regulate Dendritic Spine Morphogenesis, *Cell Rep.* 18 (2017) 2702–2714. doi:10.1016/j.celrep.2017.02.060.
- [109] M. Yamagata, X. Duan, J.R. Sanes, Cadherins Interact With Synaptic Organizers to Promote Synaptic Differentiation, *Front. Mol. Neurosci.* 11 (2018) 142. doi:10.3389/fnmol.2018.00142.
- [110] S.D. Santos, M.J. Saraiva, Enlarged ventricles, astrogliosis and neurodegeneration in heat shock factor 1 null mouse brain, *Neuroscience*. 126 (2004) 657–663. doi:10.1016/j.neuroscience.2004.03.023.
- [111] E. Takaki, M. Fujimoto, T. Nakahari, S. Yonemura, Y. Miyata, N. Hayashida, K. Yamamoto, R.B. Vallee, T. Mikuriya, K. Sugahara, H. Yamashita, S. Inouye, A. Nakai, Heat Shock Transcription Factor 1 Is Required for Maintenance of Ciliary Beating in Mice, *J. Biol. Chem.* 282 (2007) 37285–37292. doi:10.1074/jbc.M704562200.

- [112] X. Cui, J. Zhang, R. Du, L. Wang, S. Archacki, Y. Zhang, M. Yuan, T. Ke, H. Li, D. Li, C. Li, D.W.-C. Li, Z. Tang, Z. Yin, M. Liu, HSF4 is involved in DNA damage repair through regulation of Rad51, *Biochim. Biophys. Acta.* 1822 (2012) 1308–1315. doi:10.1016/j.bbadis.2012.05.005.
- [113] X. Cui, H. Liu, J. Li, K. Guo, W. Han, Y. Dong, S. Wan, X. Wang, P. Jia, S. Li, Y. Ma, J. Zhang, H. Mu, Y. Hu, Heat shock factor 4 regulates lens epithelial cell homeostasis by working with lysosome and anti-apoptosis pathways, *Int. J. Biochem. Cell Biol.* 79 (2016) 118–127. doi:10.1016/j.biocel.2016.08.022.
- [114] M. Fujimoto, R. Takii, E. Takaki, A. Katiyar, R. Nakato, K. Shirahige, A. Nakai, The HSF1-PARP13-PARP1 complex facilitates DNA repair and promotes mammary tumorigenesis, *Nat. Commun.* 8 (2017) 1638. doi:10.1038/s41467-017-01807-7.
- [115] S. Homma, X. Jin, G. Wang, N. Tu, J. Min, N. Yanasak, N.F. Mivechi, Demyelination, Astrogliosis, and Accumulation of Ubiquitinated Proteins, Hallmarks of CNS Disease in hsf1-Deficient Mice, *J. Neurosci.* 27 (2007) 7974–7986. doi:10.1523/JNEUROSCI.0006-07.2007.
- [116] X. Shi, B. Cui, Z. Wang, L. Weng, Z. Xu, J. Ma, G. Xu, X. Kong, L. Hu, Removal of Hsf4 leads to cataract development in mice through down-regulation of γ S-crystallin and Bfsp expression, *BMC Mol. Biol.* 10 (2009) 10. doi:10.1186/1471-2199-10-10.
- [117] H. Izu, S. Inouye, M. Fujimoto, K. Shiraishi, K. Naito, A. Nakai, Heat Shock Transcription Factor 1 Is Involved in Quality-Control Mechanisms in Male Germ Cells, *Biol. Reprod.* 70 (2004) 18–24. doi:10.1095/biolreprod.103.020065.
- [118] L. Pignataro, A.N. Miller, L. Ma, S. Midha, P. Protiva, D.G. Herrera, N.L. Harrison, Alcohol regulates gene expression in neurons via activation of heat shock factor 1, *J. Neurosci. Off. J. Soc. Neurosci.* 27 (2007) 12957–12966. doi:10.1523/JNEUROSCI.4142-07.2007.
- [119] L. Pignataro, F.P. Varodayan, L.E. Tannenholz, P. Protiva, N.L. Harrison, Brief alcohol exposure alters transcription in astrocytes via the heat shock pathway, *Brain Behav.* 3 (2013) 114–133. doi:10.1002/brb3.125.
- [120] S. Ishii, M. Torii, A.I. Son, M. Rajendraprasad, Y.M. Morozov, Y.I. Kawasawa, A.C. Salzberg, M. Fujimoto, K. Brennand, A. Nakai, V. Mezger, F.H. Gage, P. Rakic, K. Hashimoto-Torii, Variations in brain defects result from cellular mosaicism in the activation of heat shock signalling, *Nat. Commun.* 8 (2017) 15157. doi:10.1038/ncomms15157.
- [121] A.L. Schang, D. Sabéran-Djoneidi, V. Mezger, The impact of genomic and epigenomics NGS approaches on our understanding of neuropsychiatric disorders, *Clin. Genet.* (2017).
- [122] Y. Hakak, J.R. Walker, C. Li, W.H. Wong, K.L. Davis, J.D. Buxbaum, V. Haroutunian, A.A. Fienberg, Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia, *Proc. Natl. Acad. Sci.* 98 (2001) 4746–4751. doi:10.1073/pnas.081071198.
- [123] D. Arion, T. Unger, D.A. Lewis, P. Levitt, K. Mirnics, Molecular evidence for increased expression of genes related to immune and chaperone function in the prefrontal cortex in schizophrenia, *Biol. Psychiatry.* 62 (2007) 711–721. doi:10.1016/j.biopsych.2006.12.021.
- [124] W. Hennah, P. Thomson, A. McQuillin, N. Bass, A. Loukola, A. Anjorin, D. Blackwood, D. Curtis, I.J. Deary, S.E. Harris, E.T. Isometsä, J. Lawrence, J. Lönngqvist, W. Muir, A. Palotie, T. Partonen, T. Paunio, E. Pylkkö, M. Robinson, P. Soronen, K. Suominen, J. Suvisaari, S. Thirumalai, D. St Clair, H. Gurling, L. Peltonen, D. Porteous, DISC1 association, heterogeneity and interplay in schizophrenia and bipolar disorder, *Mol. Psychiatry.* 14 (2009) 865–873. doi:10.1038/mp.2008.22.
- [125] P.F. Sullivan, The genetics of schizophrenia, *PLoS Med.* 2 (2005) e212. doi:10.1371/journal.pmed.0020212.
- [126] H. Hagberg, P. Gressens, C. Mallard, Inflammation during fetal and neonatal life: implications for neurologic and neuropsychiatric disease in children and adults, *Ann. Neurol.* 71 (2012) 444–457. doi:10.1002/ana.22620.
- [127] M. Lin, D. Zhao, A. Hrabovsky, E. Pedrosa, D. Zheng, H.M. Lachman, Heat shock alters the expression of schizophrenia and autism candidate genes in an induced pluripotent stem cell

- model of the human telencephalon, *PLoS One*. 9 (2014) e94968. doi:10.1371/journal.pone.0094968.
- [128] R.M. Fame, C. Dehay, H. Kennedy, J.D. Macklis, Subtype-Specific Genes that Characterize Subpopulations of Callosal Projection Neurons in Mouse Identify Molecularly Homologous Populations in Macaque Cortex, *Cereb. Cortex N. Y. N* 1991. 27 (2017) 1817–1830. doi:10.1093/cercor/bhw023.
- [129] F. García-Moreno, Z. Molnár, Subset of early radial glial progenitors that contribute to the development of callosal neurons is absent from avian brain, *Proc. Natl. Acad. Sci. U. S. A.* 112 (2015) E5058-5067. doi:10.1073/pnas.1506377112.
- [130] S. Aivazidis, C.M. Coughlan, A.K. Rauniyar, H. Jiang, L.A. Liggett, K.N. Maclean, J.R. Roede, The burden of trisomy 21 disrupts the proteostasis network in Down syndrome, *PLoS One*. 12 (2017) e0176307. doi:10.1371/journal.pone.0176307.
- [131] R.A. Veitia, S. Bottani, J.A. Birchler, Cellular reactions to gene dosage imbalance: genomic, transcriptomic and proteomic effects, *Trends Genet. TIG*. 24 (2008) 390–397. doi:10.1016/j.tig.2008.05.005.
- [132] A. Duchon, Y. Herault, DYRK1A, a Dosage-Sensitive Gene Involved in Neurodevelopmental Disorders, Is a Target for Drug Development in Down Syndrome, *Front. Behav. Neurosci.* 10 (2016) 104. doi:10.3389/fnbeh.2016.00104.
- [133] T. Gidalevitz, A. Ben-Zvi, K.H. Ho, H.R. Brignull, R.I. Morimoto, Progressive disruption of cellular protein folding in models of polyglutamine diseases, *Science*. 311 (2006) 1471–1474. doi:10.1126/science.1124514.
- [134] T. Gidalevitz, E.A. Kikis, R.I. Morimoto, A cellular perspective on conformational disease: the role of genetic background and proteostasis networks, *Curr. Opin. Struct. Biol.* 20 (2010) 23–32. doi:10.1016/j.sbi.2009.11.001.
- [135] S.L. Rutherford, S. Lindquist, Hsp90 as a capacitor for morphological evolution, *Nature*. 396 (1998) 336–342. doi:10.1038/24550.
- [136] D.F. Jarosz, S. Lindquist, Hsp90 and environmental stress transform the adaptive value of natural genetic variation, *Science*. 330 (2010) 1820–1824. doi:10.1126/science.1195487.
- [137] V. Mezger, O. Bensaude, M. Morange, Unusual levels of heat shock element-binding activity in embryonal carcinoma cells, *Mol. Cell. Biol.* 9 (1989) 3888–3896. doi:10.1128/mcb.9.9.3888.
- [138] M. Fujimoto, H. Izu, K. Seki, K. Fukuda, T. Nishida, S. Yamada, K. Kato, S. Yonemura, S. Inouye, A. Nakai. HSF4 is required for normal cell growth and differentiation during mouse lens development. *EMBO J.* 2004 Oct 27;23(21):4297-306. Epub 2004 Oct 14. doi: 10.1038/sj.emboj.7600435