# Stress Management: Multilayer Regulation of the Heat Shock Response

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*"For science is more than the search for truth, more than a challenging game, more than a profession. It is a life that a diversity of people lead together, in the closest proximity, a school for social living. We are members one of another."*

A. G. Ogston

## ABSTRACT

Protein-damaging stresses, such as exposure to elevated temperature, alcohol, and heavy metals and microbial infections, increase the amount of misfolded proteins in cells, which may lead to formation of toxic protein aggregates. In order to counteract detrimental effects of proteotoxic stresses, all organisms have developed a specific response, called the heat shock response (HSR). Upon the HSR activation, synthesis of heat shock proteins (HSPs) increases. HSPs function as molecular chaperones to aid in the refolding of misfolded proteins and prevent formation of protein aggregates. The inducible expression of HSP genes is mediated by heat shock factors (HSFs). Mounting the HSR involves trimerization, nuclear accumulation, gain of DNA-binding and *trans*-activation capacity of HSF1 and coincides with extensive post-translational modifications of HSF1. However, the importance of these modifications on HSF1 activity has remained elusive. This PhD thesis describes the functional impact of stress-inducible phosphorylation of HSF1 on the HSR. While HSF1 phosphorylation upon stress is not required for the HSR activation, it defines the onset of the HSR and fine-tunes the magnitude of the response. Moreover, a chaperone co-inducer BGP-15 was found to augment the HSR, due to its ability to inhibit histone deacetylases (HDACs). BGP-15 as an HDAC inhibitor increases chromatin accessibility at multiple genomic loci including HSP genes and accelerates HSF1 activation in the event of proteotoxic stress. Furthermore, HDAC inhibitors can function as chaperone co-inducers and have cytoprotective effect for cells exposed to stress. Taken together, this study provides valuable information on molecular mechanisms regulating the HSR, which can serve as a basis for further development of pharmaceuticals aimed to activate, enhance or repress the HSR.

## SAMMANFATTING (Swedish Abstract)

Cellens överlevnad är beroende av förmågan att upprätthålla proteiners struktur och funktion. De cellulära proteinerna är uppbyggda av aminosyrakedjor och de är essentiella för nästan varje uppgift av cellulärt liv. För att kunna överleva cellulär stress som skadar proteiners struktur, aktiverar cellen specifika försvarsmekanismer. Förändringar i den intracellulära eller extracellulära omgivningen, vilka skadar proteostasen, aktiverar den evolutionärt konserverade försvarsmekanismen som kallas för värmechockresponsen (eng. heat shock response, HSR). Aktiveringen av HSR leder till en markant ökning i uttryck av värmechockproteiner (eng. heat shock proteins, HSPs). HSP:er är molekylära chaperoner som hjälper andra proteiner att upprätthålla sin struktur och funktion. Molekylära chaperoner kan dessutom hindra formationen av toxiska proteinaggregat och i situationer där proteiners struktur är allvarligt skadade kan HSP:er rikta dem för nedbrytning. Under HSR är uttrycket av HSP-generna strikt reglerade av värmechockfaktorer (eng. heat shock factors, HSFs). I däggdjur är HSF1 den huvudsakliga regleraren av HSR. Vid cellulär stress bildar HSF1 trimerer, vilka i sin tur ansamlas i cellkärnan där de binder till promotorregioner vid sina målgener. Under aktiverings-attenueringsräckan av HSR genomgår HSF1 omfattande posttranslationella modifieringar, såsom fosforylering. Det är dock okänt i vilken grad dessa ändringar påverkar HSF1:s transaktiveringskapacitet. Stressinducerbar fosforylering har ansetts krävas för aktivering av HSF1, men klara bevis för eller emot denna hypotes saknas.

Inducerad aktivering av HSR anses vara en lovande behandlingsmetod för att återställa proteinhomeostasen i sjukdomstillstånd, såsom neurodegeneration. Flera analyser har identifierat ett stort antal HSF1-aktivatorer, men många av dem ger upphov till allvarliga biverkningar, vilket avhåller deras användning i kliniker. Följaktligen betraktas så kallade chaperonkoincuderare som en alternativ metod för att öka på HSP-uttrycket i olika sjukdomstillstånd eftersom de kan inte direkt aktivera HSR. Den exakta mekanismen för deras verkan är dock ännu okänd, vilket avhåller deras utveckling som effektiva läkemedel.

I denna avhandling har jag skapat en HSF1-mutant, som saknar bindningsställen för fosfor, för att kunna studera den funktionella inverkan av fosforylering vid HSF1:s aktivering. Med hjälp av denna mutant har jag visat att stressinducerbar fosforylering inte är nödvändigt för HSF1:s aktivering, men att den istället styr tröskelvärdet för dess aktivering. Dessutom visar jag i denna avhandling att chaperonkoinduceraren BGP-15 ökar på uttrycket av HSP:er under cellulära stresser genom att öppna kromatinet. Denna egenskap beror på att BGP-15 kan inhibera histondeacetylaser (eng. histone deacetylases, HDACs). BGP-15 kan dessutom påverka tröskelvärdet för HSF1:s aktivering,

vilket möjliggör HSF1:s aktivering under stressförhållanden som normalt inte skulle aktivera HSR. Dessa resultat visar att chaperonkoinducerare kan förstärka HSR och därmed öka på cellens förmåga att hantera proteinskadande stress.

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## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publication and a manuscript, which are referred to in the text by Roman numerals (I-II). The original publication has been reproduced with permission of the copyright holder.

**I.** Budzyński M.A., Puustinen M.C., Joutsen J., Sistonen L. (2015) Uncoupling Stress-Inducible Phosphorylation of Heat Shock Factor 1 from Its Activation. *Mol. Cell. Biol.* 35: 2530-2540. doi: 10.1128/MCB.00816-14

**II.** Budzyński M.A.*, Crul T.*, Himanen S., Toth N., Otvos F., Sistonen L., Vigh L. Chaperone Co-inducer BGP-15 Inhibits Histone Deacetylases and Enhances the Heat Shock Response through Increased Chromatin Accessibility. Submitted manuscript.

* equal contribution

ADDITIONAL PUBLICATION NOT INCLUDED IN THESIS

Budzyński M.A., Sistonen L. Versatile Functions of Heat Shock Factors: It Is Not All about Stress. Manuscript accepted to *Curr. Immunol. Rev.*, in press. doi: 10.2174/1573395513666170316110147

## ABBREVIATIONS

| | |
|---|---|
| AD | Activatory domain |
| ATP | Adenosine 5′-triphosphate |
| CDK | Cyclin-dependent kinase |
| ChIP | Chromatin immunoprecipitation |
| CNS | Central nervous system |
| CTD | C-terminal domain |
| DBD | DNA-binding domain |
| EMSA | Electrophoretic mobility shift assay |
| ERK | Extracellular signal–regulated kinases |
| Gal4 | Galactose-responsive transcription factor GAL4 |
| GTF | General transcription factor |
| HAT | Histone acetyltransferase |
| HD | Huntington's disease |
| HDAC | Histone deacetylase |
| HR-A/B/C | Heptad repeat A/B/C domain |
| HSE | Heat shock element |
| HSF | Heat shock factor |
| HSP | Heat shock protein |
| HSR | Heat shock response |
| ILS | Insulin-like signaling |
| JNK | c-Jun N-terminal kinase |
| MAPK | Mitogen-activated protein kinase |
| MEF | Mouse embryonic fibroblast |
| MEK | Mitogen-activated protein kinase kinase |
| MNase | Micrococcal nuclease |
| mTORC | Mammalian target of rapamycin complex |
| NAD | Nicotinamide amide dinucleotide |
| NLS | Nuclear localization signal |
| nSB | Nuclear stress body |
| p53 | Tumor protein p53 |

| | |
|---|---|
| PDSM | Phosphorylation-dependent sumoylation motif |
| PIC | Preinitiation complex |
| PKC | Protein kinase C |
| Pol II | RNA polymerase II |
| P-TEFb | Positive transcription elongation factor b |
| PTM | Post-translational modification |
| RD | Regulatory domain |
| SIRT | Sirtuin |
| TBP | TATA-binding protein |
| TPA | Phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate |
| TSA | Trichostatin A |
| TSS | Transcription start site |
| UPR | Unfolded protein response |
| VP16 | Herpes simplex virus protein vmw65 |
| VPA | Valproic acid |

# INTRODUCTION

Each cell maintains a delicate balance of proteins to sustain their function. To cope with cellular stresses, an appropriate protective response is mounted, which ensures the cell's survival. Proteins built from the amino acids are cellular workhorses and responsible for nearly every task of the cellular life. Changes in the intracellular or extracellular environment that prevent the acquisition of normal protein structure activate an evolutionarily conserved response known as the heat shock response (HSR). Mounting the HSR leads to inducible expression of heat shock proteins (HSPs). HSPs function as molecular chaperones, and bind to the misfolded proteins and assist in their refolding to prevent the formation of toxic protein aggregates. If the damage is too severe, HSPs direct damaged proteins to degradation. In eukaryotes expression of HSP genes during the HSR is strictly regulated by heat shock factors (HSFs). In mammals, among the HSF family members, HSF1 is a master regulator of the HSR. Upon proteotoxic stresses, HSF1 forms trimers and accumulates into the nucleus, where it regulates transcription by binding to either promoters or enhancers of its target genes. During its activation-attenuation pathway, HSF1 undergoes extensive post-translational modifications, such as phosphorylation. However, it is unknown to what extent these modifications affect the *trans*-activation capacity of HSF1. Stress-inducible phosphorylation is thought to be required for HSF1 activation, but compelling evidence for or against that hypothesis is missing.

Activation of the HSR is perceived as a promising therapeutic strategy for restoring protein homeostasis in various proteinopathies, such as neurodegeneration. Multiple screens identified a large number of HSF1 activators, but many of them yield unwanted side-effects, *i.e.* they induce protein damage, which severely hamper their progress into the clinics. Therefore, chaperone co-inducers are considered as another approach to increasing HSP levels in proteinopathies. While not being able to activate the HSR directly, chaperone co-inducers enhance expression of HSPs in the event of protein-damaging stimuli. Nevertheless, the exact mechanism of their action is scarce, which impedes their development as effective pharmaceuticals.

In this thesis, I have generated a phosphorylation-deficient HSF1 mutant to study the functional impact of phosphorylation on HSF1 activation. Using this mutant, I was able to established that stress-inducible phosphorylation of HSF1 is not required for activation of HSF1-mediated HSR. Phosphorylation plays, however, an important role in the HSR as it defines the threshold for HSF1 activation under proteotoxic stress conditions, as wells as fine-tunes the HSR. Furthermore, this thesis shows that a chaperone co-inducer BGP-15 increases HSPs expression upon stress through chromatin opening. This property of BGP-15 is due to its ability to inhibit histone deacetylases (HDACs). BGP-15 also sensitizes HSF1, allowing its activation under stress conditions that normally would not cause the HSR activation. These results position HDACs as potent chaperone co-inducers, capable of enhancing the HSR and improving cytoprotection upon proteotoxic stress.

# REVIEW OF THE LITERATURE

## 1. Cellular stress responses

Every living thing on our planet is composed of the smallest building block, which is the cell. Most living organisms are single cells, but many others including humans are multicellular organisms, with cells performing specialized functions, forming tissues, and are linked by a complex system of communication. Each cell is a small universe of its own. The eukaryotic cells contain all the genetic information of the organism encoded in the form of the DNA in a structure called the nucleus. In addition to the nucleus, eukaryotic cells contain several other types of organelles, such as mitochondria, chloroplasts, the endoplasmic reticulum, the Golgi apparatus, and lysosomes. Each of these organelles performs specific functions critical to the cell's survival. Proteins built from the amino acids are cellular workhorses and are responsible for nearly every aspect of cellular life, including cell shape and inner cellular organization, energy production, synthesis of new proteins, and waste cleanup.

Each cell maintains a fragile balance of its components to sustain their function (homeostasis). Cellular homeostasis is in danger when cells are exposed to various forms of stresses. To cope with cellular stresses, an appropriate protective response is mounted, which ensures the cell's survival. Failure to activate a protective response or exposure to a severe stress leads to activation of signaling cascades that result in the death of the cell (reviewed in Fulda *et al.*, 2010). Depending on the type of stress and the site of damage, distinct stress response pathways are activated (**Figure 1**).

General damage to the proteins caused by agents, such as heat, heavy metals, ethanol, proteasome inhibitors, or microbial infections induces a highly conserved program called the heat shock response (HSR) (reviewed in Lindquist, 1986; and in Morimoto, 2011). Activation of the HSR *via* its master regulator the heat shock factor 1 (HSF1) leads to selective expression of heat shock proteins (HSPs). The HSR will be discussed in more detail in Chapter 2., Heat Shock Response.

### 1.1. Hypoxic Stress Response

Aerobic organisms require oxygen ($O_2$), which is an essential nutrient that serves as a key substrate in cellular metabolism and bioenergetics. During low $O_2$ (hypoxic) conditions, cells activate a number of adaptive responses (Hypoxia Stress Response), to match $O_2$ supply with metabolic, bioenergetic, and redox demands. The transcriptional program activated during hypoxic conditions is regulated by hypoxia-inducible factors (HIFs) (reviewed in Carmeliet *et al.*, 1998). In a well-oxygenated environment, HIF-1α is hydroxylated by prolyl hydroxylase domain-containing (PHD) enzymes, which leads to interaction with E3 ubiquitin ligase, the von Hippel-Lindau tumor suppressor protein (pVHL) followed by the proteasomal destruction of HIF-1α (Ivan *et al.*, 2001; Jaakkola *et al.*, 2001; Ratcliffe *et al.*, 1999; Salceda and Caro, 1997). During hypoxia, PHD activity is diminished, which stabilizes HIF-1α allowing

its translocation into the nucleus (Berra *et al.*, 2001). In the nucleus, HIF-1α heterodimers bind to hypoxia response elements (HREs) in the genome (Miyazaki *et al.*, 2002). HIF-1α directly drives the expression of genes that control the shift from oxidative to glycolytic metabolism and inhibition of cellular proliferation (Benita *et al.*, 2009). Furthermore, HIF-1α has been shown to regulate the activity of other transcriptional regulators, such as NFκB and p53 (Benita *et al.*, 2009). This demonstrates the complexity of the transcriptional response during hypoxic conditions, which involves primary and secondary changes in gene expression.

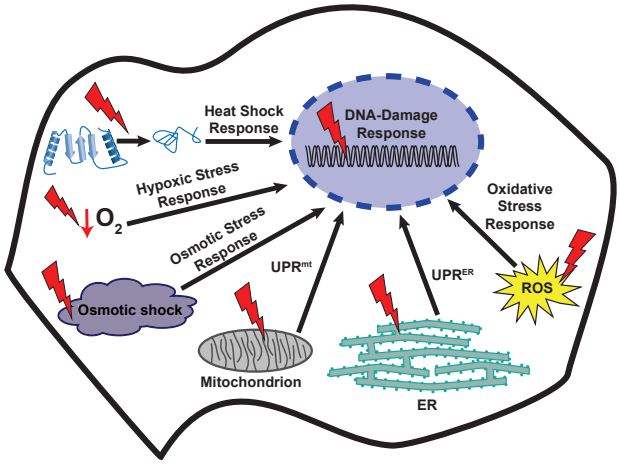


**Figure 1. Cellular stress responses.** To maintain their function in the events of stress, cells activate specific transcriptional programs. Various protein damaging stresses, such as elevated temperature, ethanol, and heavy metals exposure, and microbial infection activate the heat shock response. When the cell encounters insufficient $O_2$ availability, the hypoxia stress response is activated. The influx of water from (hyperosmotic stress) or into (hypoosmotic stress) the cell, results in mounting the osmotic stress response. Protein misfolding in specific organelles activates the unfolded protein response (UPR), in mitochondria UPR$^{mt}$, in endoplasmatic reticulum UPR$^{ER}$. Uncontrolled increase in reactive oxygen species (ROS) turns on the oxidative stress response.

### 1.2. Osmotic Stress Response

Tissues in the organism maintain specific osmotic conditions to maintain their functions. For example, kidneys and the components of blood supply exist in the hyperosmotic state (Burg *et al.*, 2007). Changes in extracellular osmolarity affect cell volume, and therefore, the concentration of intracellular macromolecules. Osmotic stress leads to efflux or influx of water from or into the cell: hyperosmotic stress causes cell shrinking, while the hypoosmotic stress causes swelling of the cell. When cells are exposed to hyperosmotic stress, activity of osmolytes and membrane transporter increases along with the activity of aldose reductase (AR), which synthesizes sorbitol, one of the main osmolytes in the cell (Uchida *et al.*, 1991). This increase is achieved *via* transcriptional regulation of the *cis*-element named tonicity-responsive enhancer (TonE) (Miyakawa *et al.*, 1998). TonE binding protein (TonEBP) was later identified as a transcriptional regulator of genes involved in the hyperosmotic stress response

(Miyakawa *et al.*, 1999). Interestingly, other groups have cloned the same protein and named it alternatively as NFAT5 (nuclear factor of activated T cell 5), NFATL1 (NFAT-like protein 1) or OREBP (osmotic response element binding protein) (Ko *et al.*, 2000; Lopez-Rodríguez *et al.*, 1999; Trama *et al.*, 2000). Activated TonEBP undergoes phosphorylation and drives expression of multiple genes, such as AR, HSPA1B, HSPB5, and sodium/chloride/betaine cotransporter (BGT1), therefore, allowing cellular adaptation to hyperosmotic stress (Lee *et al.*, 2011). Response to hypoosmotic stress is mainly regulated by the expression and activity of the aquaporins, water channels located on the plasma membrane (reviewed in Agre *et al.*, 2002). Moreover, during hypoosmotic stress the activity of TonEBP decreases (Ferraris *et al.*, 2002) and transcription of genes other than aquaporin increases, such as Egr-1, a zinc finger-containing transcription factor (Zhang and Cohen, 1997). However, to date, the specific transcriptional program during hypoosmosis is not fully characterized. Furthermore, it remains to be elucidated whether a single transcription activator regulates transcriptional changes in the events of hypoosmosis, or multiple transcription factors are involved.

### 1.3. Unfolded Protein Response

The endoplasmic reticulum (ER) is an organelle, where all the transmembrane and secreted proteins are folded and undergo post-translational modifications (PTMs) (reviewed in Braakman and Hebert, 2013). The ER is also a site of lipid production for most of the membrane-enclosed organelles and serves as an intracellular storage of $Ca^{2+}$. In order to perform its function, a specific environment inside the ER is maintained, which differs from that in the cytosol. Exposure of cells to conditions such as glucose starvation, inhibition of protein glycosylation, disturbance of $Ca^{2+}$ homeostasis, and oxygen deprivation causes accumulation of unfolded and misfolded proteins in the ER resulting in activation of the unfolded protein response in the ER ($UPR^{ER}$) (reviewed in Schröder and Kaufman, 2005). $UPR^{ER}$ activation is mediated by three transmembrane sensors: the inositol requiring element-1 (IRE-1), the PKR-like ER kinase (PERK) and the activating transcription factor 6 (ATF6). Under normal conditions, IRE-1, PERK and ATF6 are kept inactive by an ER chaperone BiP (also known as HSPA5 or GRP78) (Bertolotti *et al.*, 2000; Liu *et al.*, 2003; Shen *et al.*, 2002). The increase of unfolded and misfolded proteins in the ER titrates BiP away, allowing for activation of $UPR^{ER}$ mediators. Activated PERK phosphorylates the translation initiation factor 2 (eIF2a), preventing protein synthesis and influx of newly synthesized proteins into ER (Harding *et al.*, 1999). Stress-induced eIF2$\alpha$ phosphorylation also increases translation of the activating transcription factor 4 (ATF4) (Lu *et al.*, 2004). ATF6 released from the ER membrane translocates to the Golgi apparatus, where it is cleaved by proteases to form an active transcription factor (Shen *et al.*, 2002). Activated IRE1 endonucleolytically cleaves mRNA, which results in production of a splicing variant of transcription factor X-box binding protein-1 (XBP1), which encodes a protein with increased transcriptional activation potential (Cox and Walter, 1996; Yoshida *et al.*, 2001). Altogether, ATF4, ATF6, and XBP-1 drive the transcription of the $UPR^{ER}$

target genes, such as glucose-regulated proteins, to which belong ER chaperones, such as BiP and oxidoreductases (Calfon *et al.*, 2002; Lee *et al.*, 2003; Yoshida *et al.*, 1998; Yoshida *et al.*, 2001). If the stress is too severe, or the $UPR^{ER}$ malfunctions, cells undergo ER stress-induced apoptosis. ER stress-induced cell death has been shown to rely on the activation of caspase-12 in rodents and caspase-4 in humans (Hitomi *et al.*, 2004; Nakagawa *et al.*, 2000). Activation of caspase-12 leads to a non-canonical, cytochrome c-independent cleavage of procaspase-9 (Rao *et al.*, 2002). Additionally, C/EBP homologous protein (CHOP), a transcription factor that is induced downstream of the PERK and ATF6 pathways, initiates ER stress-induced cell death (McCullough *et al.*, 2001). IRE1 also participates in ER stress-induced cell death by activating c-Jun N-terminal kinase (JNK) (Urano *et al.*, 2000). JNK phosphorylates BCL2 in the ER, which suppresses the anti-apoptotic activity of BCL2 and leads to subsequent activation of caspases (Davis, 2000).

Mitochondria act as cellular power plants, producing energy that allows cells to operate. Aerobic respiration, provided by the mitochodria generates 15 times greater amount of energy compared with anaerobic respiration, and it is believed to be one of the factors which allowed development of complex, multicellular organisms (van der Giezen, 2011). Mitochondria have a matrix, which differs from the cytosol similar to the ER. Accumulation of misfolded proteins in mitochondria activates the unfolded protein response in mitochondria ($UPR^{mt}$), which involves the transcription factors CHOP and CCAAT/enhancer-binding protein (C/EBPb) (Zhao *et al.*, 2002). Activation of CHOP and C/EBPb drive the expression of nuclear genes encoding mitochondrial chaperones, such as HSPD1, HSPE1, and DNAJA2 as well as mitochondrial protease ClpP. Interestingly, CHOP is also induced by the $UPR^{ER}$, however, activation of $UPR^{mt}$ does not result in upregulation of genes involved in the $UPR^{ER}$ (Zhao *et al.*, 2002). This indicates that the $UPR^{ER}$ and $UPR^{mt}$ pathways are autonomous despite sharing signaling components.

### 1.4. Oxidative Stress Response

Reactive oxygen species (ROS) are natural byproducts of mitochondrial aerobic respiration. ROS play an important role during physiological conditions as signaling molecules, regulating autophagy, differentiation, and immunity (Rhee *et al.*, 2000; reviewed in Sena and Chandel, 2012). However, due to their ability to cause DNA and protein damage, cells keep ROS levels under strict control through various antioxidant enzymes, such as catalases, peroxidases, and superoxide dismutase (SOD) (Kirkman *et al.*, 1999; Loew, 1900). ROS overproduction or mismanagement cause oxidative stress. To counteract and minimize damage caused by ROS, cells activate a specific stress response, the oxidative stress response. Nuclear factor (erythroid-derived 2)-like factor 2 (Nrf2, also called NFE2L2) is a transcription factor that regulates the cellular redox status (Itoh *et al.*, 1997). Under normal conditions, Nrf2 undergoes proteasomal degradation in an E3 ubiquitin ligase Keap1-dependant manner (McMahon *et al.*, 2003). In the presence of ROS, Keap1 is modified at several reactive cysteine residues, which prevents Keap1-Nrf2 interaction and subsequently leads to stabilization of Nrf2

(Zhang and Hannink, 2003). Nrf2, in turn, translocates into the nucleus where it forms heterodimers with small Maf proteins and recognizes antioxidant response element (ARE)/electrophile response element (EpRE) and activates gene expression (Itoh *et al.*, 1997). A majority of genes containing ARE encode either antioxidant enzymes (SOD, catalase, peroxidases, GST-transferases) or enzymes involved in biosynthesis of antioxidants like gamma-glutamylcysteine synthetase, a key enzyme of glutathione biosynthesis (Itoh *et al.*, 1997; Rushmore *et al.*, 1991).

## 2. Heat Shock Response

Discovery of the heat shock response (HSR) dates back to the year 1962, when an Italian geneticist, Ferruccio Ritossa, found that *Drosophila buscki* larvae exhibit a specific puffing pattern of the polytene chromosomes when exposed to elevated temperature (Ritossa, 1962). It was known that these puffs are sites of RNA synthesis. It took another 12 years to establish that this puffing resulted in the production of proteins, which are now known as heat shock proteins (HSPs) (Tissières *et al.*, 1974). HSPs are a conserved protein family and have been found in all cells of all organisms (Hartl and Hayer-Hartl, 2002). HSPs, which act as molecular chaperones, help proteins to fold correctly, maintain their proper conformation, and prevents the formation of protein aggregates (reviewed in Hartl *et al.*, 2011). The HSR can be activated not only by heat stress, but also by a plethora of other protein-damaging conditions, such as oxidative stress, heavy metals, alcohol, proteasome inhibition, and microbial infections (reviewed in Morimoto, 2011). Mapping of the promoter regions of HSP genes led to the identification of a common regulatory element, the heat shock element (HSE), consisting of inverted pentameric nGAAn repeats (Amin *et al.*, 1988; Sorger and Pelham, 1988). These findings allowed, in turn, for cloning and characterization of an HSE-binding protein, named heat shock factor 1 (HSF1) (Rabindran *et al.*, 1991; Wiederrecht *et al.*, 1987; Wu *et al.*, 1987). HSF1 is a central player in the HSR (**Figure 2**). In the classical HSR model, protein-damaging stresses cause accumulation of misfolded proteins followed by HSF1 activation. HSF1 drives inducible expression of HSP genes, which results in accumulation of HSPs. Newly synthesized HSPs bind to misfolded proteins to alleviate the damage. In this Chapter, I will discuss general mechanisms of gene expression, how the chromatin landscape can regulate transcription, classification, and function of HSPs, mechanisms governing HSR activation, and pharmacological strategies for controlling HSR.

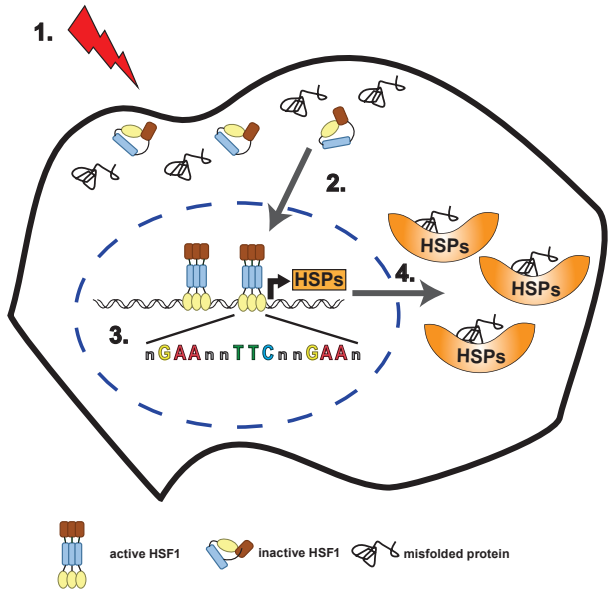


**Figure 2. The Heat Shock Response. 1.** Protein-damaging stresses lead to accumulation of misfolded and unfolded proteins within the cell. **2.** Heat shock factor 1 (HSF1) becomes activated, forms trimers, and accumulates in the nucleus. **3.** Active HSF1 binds to heat shock elements, DNA sequences containing inverted pentameric nGAAn repeats, at the promoters of its target genes, mainly heat shock proteins (HSPs). HSF1 activates stress-inducible expression of HSPs. **4.** Newly synthesized HSPs bind to misfolded and unfolded proteins facilitating their refolding or target them to proteasomal degradation.

### 2.1. *HSP70* as a model gene for studying inducible transcription

Each cell in an organism contains the same genetic information encoded in the DNA, which is composed of four nucleotides adenine (A), thymine (T), cytosine (C), and guanine (G). The smallest functional parts of DNA are called genes, which contain all the necessary information required for the synthesis of a functional product (Gerstein *et al.*, 2007). When the gene is activated, information stored in the form of a specific nucleotide sequence is copied (transcribed) by a multi-subunit protein complex RNA polymerase II (Pol II) into a messenger RNA (mRNA) molecule. In turn, mRNA serve as a template for synthesis of proteins. The process by which information written in RNA gives rise to a protein is called translation and occurs in the ribosomes in the cytoplasm.

The human genome contains approximately 21 000 protein-coding genes (Clamp *et al.*, 2007). Although most cells and tissues express a high fraction of all protein-encoding genes (~65%), their expression greatly varies from cell to cell and from tissue to tissue (Pontén *et al.*, 2009). This suggests that tissue specificity is achieved not only by controlling which genes are expressed, but also by precise regulation of how much these genes are expressed.

For decades, the *Drosophila HSP70 loci* served as a model gene for studying inducible transcription. The *HSP70* gene under normal conditions is transcribed at low frequency. However, it is transcribed several hundred folds higher in response to increased temperatures (Mosser *et al.*, 1988). Research on *HSP70* gene led to many seminal findings, such as a discovery of transcriptionally engaged, but paused Pol II at the gene promoter regions (Rougvie and Lis, 1988). Initially, the paused Pol II was thought to be *Drosophila* HSP genes specific, but further analyses have revealed that Pol II pausing is genome-wide phenomena present across metazoans (Guenther *et al.*, 2007; Kim *et al.*, 2005b; Mahat *et al.*, 2016; Muse *et al.*, 2007; Nechaev *et al.*, 2010). Below, I briefly describe the processes of the establishment and release of the paused Pol II.

The establishment of the paused Pol II involves both the formation of the preinitiation complex (PIC) at the gene promoter and retainment of the early elongation complex within the paused site. Assembly of the PIC at the transcription start site (TSS) requires key regulatory sequences, known as the core promoter, which is predominantly located ~35 base pairs (bp) upstream of the TSS (reviewed in Kadonaga, 2012). Common core promoter elements in eukaryotes include an initiator, a polypyrimidine initiator, a TATA box, downstream promoter elements (DRE), TFIIB recognition elements (BRE), and a motif ten element (MTE). TATA-containing promoters have been extensively studied. Therefore I will use it as an example to describe the formation of the PIC. The TATA-box is usually located 30 bp upstream the TSS and is composed of an AT-rich region (Goldberg, 1979). Formation of the PIC is initiated by the general transcription factors (GTFs) TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH. TFIID is the largest GTF and forms a protein complex consisting of TATA-binding protein (TBP) along with multiple TBP-associated factors (TAFs) (**Figure 3**) (Goodrich and Tjian, 1994; Komarnitsky *et al.*, 1999). TBP facilitates binding of TFIID to the TATA box, which induces DNA bending and recruits other PIC components to the promoter (Woychik and Hampsey, 2002). Although *in vitro* formation of the PIC can be achieved by the addition of the PIC components in a step-by-step manner, *in vivo* experiments show that transcriptional machinery is recruited to promoters as a holoenzyme containing Pol II, GTFs, and additional co-factors (reviewed in Thomas and Chiang, 2006). Eukaryotic DNA is organized into a structure called chromatin (reviewed in Schlick *et al.*, 2012). The principal component of chromatin is the nucleosome, where 146 bp of DNA is wrapped around a histone octamer composed of one heterotetramer of histone H3 and histone H4 as well as two heterodimers of histone H2A and H2B. Histone H1, while not a part of a core histone octamer, stabilizes the nucleosome by interacting with DNA, where it enters and exits the histone octamer. The presence of the nucleosomes in the promoter region might prevent recognition of critical sequences, by making them inaccessible. Thus, formation of the PIC requires a promoter opening, which frequently involves the binding of a sequence-specific transcription factor that recruits chromatin remodelers to make DNA accessible around the TSS (**Figure 3**) (reviewed in Workman, 2006). In *Drosophila*, GAGA factor (GAF) binds

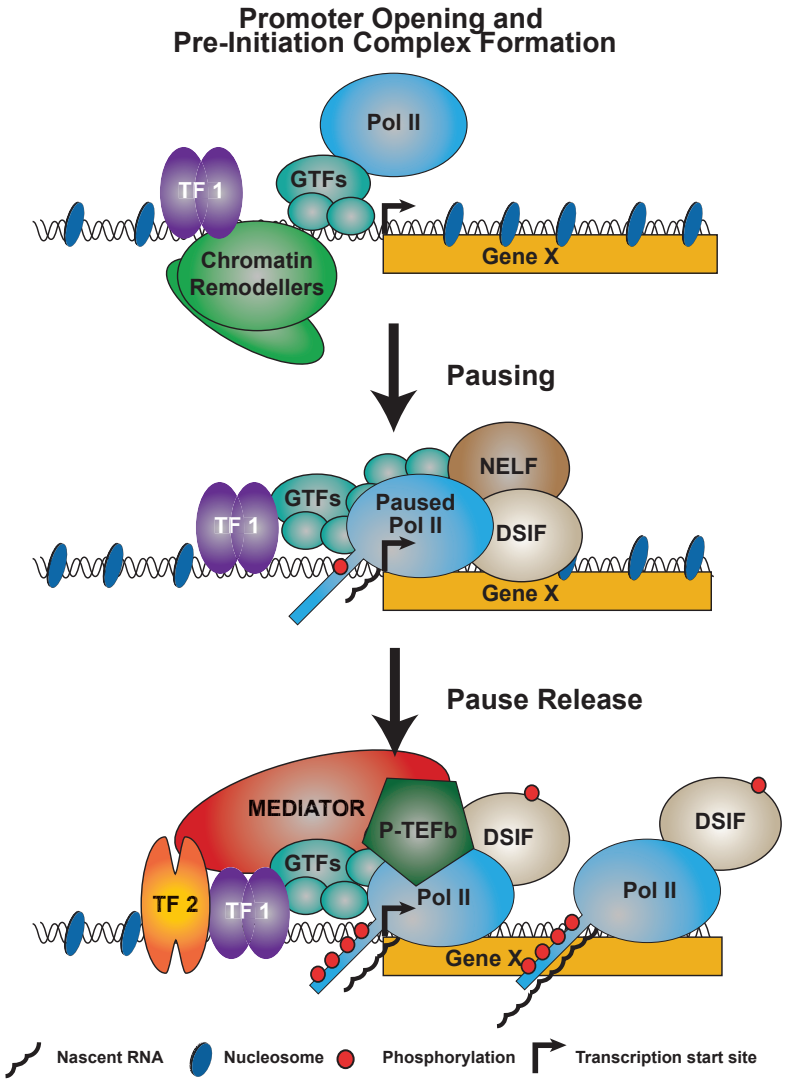


**Figure 3. Establishment and release of paused Pol II.** The promoter region is located in front of the transcription start site (TSS, marked with an arrow). The opening of the chromatin in the promoter region often requires binding of a sequence-specific transcription factor (TF1), which recruits chromatin remodelers to remove nucleosomes from around the TSS. General transcription factors (GTFs) and RNA polymerase II (Pol II) are required for formation of the preinitiation complex. Pausing of Pol II occurs after transcription initiation and transcription of 20-60 nucleotides long mRNA and involves association of pausing factors, such as negative elongation factor (NELF) and DRB sensitivity-inducing factor (DSIF). The paused Pol II is phosphorylated on its carboxy-terminal heptapeptide repeat domain (CTD). Binding of a transcription factor (TF2) to the promoter region results in recruitment of the positive transcription elongation factor (P-TEFb) kinase and other transcriptional regulators, such as the Mediator complex. P-TEFb kinase phosphorylates the DSIF–NELF complex to release paused Pol II and it also further phosphorylates S5 the Pol II CTD. Phosphorylation of DSIF–NELF dissociates NELF from the elongation complex and transforms DSIF into a positive elongation factor. Escape of the paused Pol II into productive elongation is followed by entry of another Pol II into the pause site and subsequent release. When the gene is activated, further nucleosome removal from the gene body occurs.

upstream of TATA box and keeps open the promoter of the *HSP70* gene as well as many other stress-inducible genes (Duarte *et al*., 2016; Weber *et al*., 1997). In mammals, there is no GAF homolog, however, it was proposed that HSF1 itself could maintain the *HSP70* promoter open *via* recruitment of the histone chaperone FACT and chromatin remodeling complex containing Brahma-related gene 1 ( BRG1) (Corey *et al*., 2003; Fujimoto *et al*., 2012; Sullivan *et al*., 2001).

Pol II facilitates the recruitment of proteins required for transcription and RNA processing *via* phosphorylation of its C-terminal domain (CTD) (reviewed in Hsin and Manley, 2012). Pol II CTD is composed of multiple YSPTSPS repeats, varying in number from 26 in *S. cerevisiae* to 52 in vertebrates. After formation of the PIC, the DNA is unwound, which allows transcription initiation of Pol II and promoter escape. Soon after transcription initiation Pol II is paused by DRB sensitivity-inducing factor (DSIF) and negative elongation factor (NELF), thus providing a window of opportunity for mRNA capping (Kwak and Lis, 2013). The release of the paused Pol II into productive elongation requires recruitment of the positive transcription elongation factor (P-TEFb) complex (**Figure 3**) (Marshall and Price, 1995; Wada *et al*., 1998). A subunit of the P-TEFb complex, kinase CDK9, phosphorylates the DSIF/NELF complex causing NELF to dissociate from Pol II and transform DSIF into a positive elongation factor. CDK9 also phosphorylates Pol II CTD. How is then P-TEFb recruited to the paused Pol II complex? Multiple studies have shown that DNA-binding transcription activators such as c-myc and NF-κB, are able to recruit P-TEFb to the paused Pol II complex (Barboric *et al*., 2001; Eberharter and Becker, 2002). Another study showed that MED26, a subunit of the Mediator complex, is capable of recruiting P-TEFb to facilitate the release of Pol II into productive elongation (Takahashi *et al*., 2011). P-TEFb is also rapidly recruited to the *HSP70* gene upon heat shock (Lis *et al*., 2000). However, HSF1 binding alone is not sufficient for the recruitment of P-TEFb to HSP70 *loci*, which suggests the involvement of another transcriptional co-activator. Taken together, the formation of PIC followed by recruitment of P-TEFb is critical for releasing Pol II from pausing into productive elongation. It is possible that Pol II on every gene undergoes the pausing-like state to allow mRNA capping, but the rate of P-TEFb recruitment prevents accumulation and detection of the paused Pol II (Jonkers *et al*., 2014).

### 2.2. Non-coding RNAs in HSR

The protein-coding genes account for only 2% of the human genome (Alexander *et al*., 2010). The non-coding regions for many years were thought to be non-functional and were labeled as a junk DNA. However, in the last decade, it became apparent that the non-protein-coding regions of the genome are widely expressed and are of high importance for various physiological processes (Mattick and Makunin, 2006). The group of non-coding RNAs (ncRNAs) consist of multiple RNA species, such as long non-coding RNA (lncRNA), microRNA (miRNA), and small interference RNA (siRNA).

A recent genome-wide study reported that a majority of mammalian HSF1 binding sites upon heat shock are located far from the TSS and that only a third of genes that depend on HSF1 for their stress-inducible expression have HSF1 bound at their promoters (Mahat *et al.*, 2016). This strengthens the hypothesis that HSF1 upon stress does not only regulate expression of protein-coding genes but is also involved in the expression of ncRNAs. To date, the best-studied ncRNAs regulated by HSF1 are satellite-III transcripts (SatIII) belonging to the lncRNA class (Jolly *et al.*, 2004). SatIII transcripts, which are primate specific, are coded by the satellite-III family of repetitive sequences located at the 9q12 locus in the human genome (Denegri *et al.*, 2002). HSF1-dependent, stress-inducible expression of SatIII RNA (Jolly *et al.*, 2004) occurs in the sub-nuclear structures termed nuclear stress bodies, which are formed in the event of proteotoxic stress (Sandqvist and Sistonen, 2004). Two recent studies provided information about the functional role of SatIII ncRNAs upon stress (Goenka *et al.*, 2016; Hussong *et al.*, 2016). Goenka and co-workers (2016) demonstrated that SatIII transcripts mediate heat shock-induced transcriptional repression. Furthermore, loss of SatIII transcripts resulted in increased cell death upon heat stress, which highlights the physiological importance of ncRNAs during the HSR (Goenka *et al.*, 2016). Hussong and co-workers (2016) showed that SatIII transcripts are required for proper mRNA splicing of specific genes upon heat shock conditions. Downregulation of SatIII ncRNAs upon stress led to increased intron retention in spliced mRNA (Hussong *et al.*, 2016), which often results in loss of function of the protein or give rise to a truncated protein (Braunschweig *et al.*, 2014).

miRNAs are short (20-23 nucleotides), endogenous, and single-stranded RNA molecules that act as post-transcriptional regulators of gene expression, and are involved in numerous biological processes (Bartel, 2004). Elevated temperatures lead to both downregulation and upregulation of over one hundred miRNA species in mammalian cells (Wilmink *et al.*, 2010). While the information of HSF1 in the regulation of miRNAs expression is scarce, two miRNAs have been shown to be directly regulated by HSF1, *i.e.* miR-135b (Li *et al.*, 2015) and hsa-miR-432 (Das and Bhattacharyya, 2014). Taken together, HSF1-driven transcriptional program during the HSR involves not only protein-coding genes, especially HSPs but also a wide range of ncRNAs.

### 2.3. Chromatin as a transcriptional regulator

Traditionally chromatin is divided into transcription impermeable heterochromatin and transcription favorable euchromatin, which correlates with the packing state of chromatin (reviewed in Allis and Jenuwein, 2016). In 1960's, work on histone acetylation and methylation led to a hypothesis that histone PTMs might regulate transcription (Allfrey *et al.*, 1964). It was not until 1996 that the first gene encoding nuclear transcription-associated histone acetyltransferase (HAT) was cloned and purified (Brownell *et al.*, 1996). This isolated HAT (p55) displayed a striking homology to a previously known *S. cerevisiae* transcriptional co-activator Gcn5, which provided a direct link between histone acetylation and gene activity. In the same year, the first

nuclear histone deacetylase was also discovered (HDAC) (Taunton *et al.*, 1996). Remarkably, the isolated HDAC occurred to be an ortholog of *S. cerevisiae* transcriptional repressor Rpd3. These two findings provided compelling evidence that histone acetylation is an important regulatory mechanism in gene expression. In addition to p55, other nuclear HATs have been discovered, such as CBP/p300 (CREB-binding protein/p300) (Bannister and Kouzarides, 1996), p300/CBP-associated factor (PCAF) (Ogryzko *et al.*, 1996; Yang *et al.*, 1996), and TATA-box binding protein-associated factor TFIID subunit 1 (TAF1) (Mizzen *et al.*, 1996). Currently, the mammalian family of HDACs consists of eighteen enzymes, of which eleven are zinc-dependent (class I, II, and IV), and seven require the NAD+ co-factor for activity (class III) (reviewed in Roche and Bertrand, 2016).

Histone acetylation is generally linked to active transcription (**Figure 4**). How does then histone acetylation contribute to the gene expression? One hypothesis proposes that acetylation of histones would enhance recruitment of the chromatin remodeling complexes, *e.g.* SWI/SNF, which facilitate the removal of nucleosomes subsequently increasing chromatin accessibility for transcription factors (reviewed in Tessarz and Kouzarides, 2014). Alternatively, acetylation could neutralize the basic charge in histones, thereby weakening interactions with DNA and facilitating nucleosome disassembly and chromatin opening (reviewed in Bannister and Kouzarides, 2011).

Histone methylation is another PTM which greatly affects gene expression. Histone methylation occurs on lysines and arginines, where lysines may be mono-, di- or tri-methylated, whereas arginines may be mono- or di-methylated (reviewed in Di Lorenzo and Bedford, 2011; Ng *et al.*, 2009). Histone lysines are methylated by the lysine methyl transferase (KMT), which is composed of SET-domain-containing proteins. For example, KMT G9a drives H3K9 di-methylation (Tachibana *et al.*, 2001), Polycomb and enhancer of zeste homologue 2 (EZH2) tri-methylate H3K27 (Czermin *et al.*, 2002; Kuzmichev *et al.*, 2002), while H3K4 is tri-methylated by trithorax and mixed-lineage leukaemia (MLL) (Krogan *et al.*, 2002; Milne *et al.*, 2002). Currently, a single KMT enzyme without SET domain is Dot1 which is capable of H3K79 methylation (Stulemeijer *et al.*, 2015). Histone arginines are methylated by a family of protein arginine methyltransferases (PRMTs), consisting of nine family members (Di Lorenzo and Bedford, 2011).

For many years histone methylation was considered as a stable modification. To date, two types of lysine demethylases (KDMs) have been identified, a family of enzymes containing catalytic jumonji domain (JMJD) (Tsukada *et al.*, 2005) and a single lysine-specific demethylase 1 (LSD1) (Shi *et al.*, 2004). Identification of KDMs shows that histones can undergo dynamic methylation and de-methylation. In contrast to acetylation, methylation does not change protein charge, and methylation can act as a transcriptional repressor and activator (**Figure 4**) (reviewed in Smolle and Workman, 2013). For example, H3K4 methylation marks sites with an active transcription, while methylation of H3K9 and H3K27 induces chromatin silencing. To add even more complexity, di-methylation of the same arginine residue might have either repressive

or activatory role, depending on whether it is symmetrically or asymmetrically di-methylated (reviewed in Di Lorenzo and Bedford, 2011). Methylation of histones mediates recruitment of chromatin-associated factors containing distinct domains that recognize methylated residues. These include plant homeodomain (PHD) finger domains and the Tudor "royal" family of domains, comprising of Tudor, PWWP and MBT domains (Champagne and Kutateladze, 2009; Kim *et al.*, 2006). For example, methylation of H3K4 facilitates binding of ING proteins that contain PHD finger domain. ING2 disrupts mSin3a-HDAC1 complex, promoting transcription of specific genes (Shi *et al.*, 2006). On the other hand, tri-methylation of H3K9 creates a surface for binding of HP1, potent transcriptional repressor which maintains chromatin in the

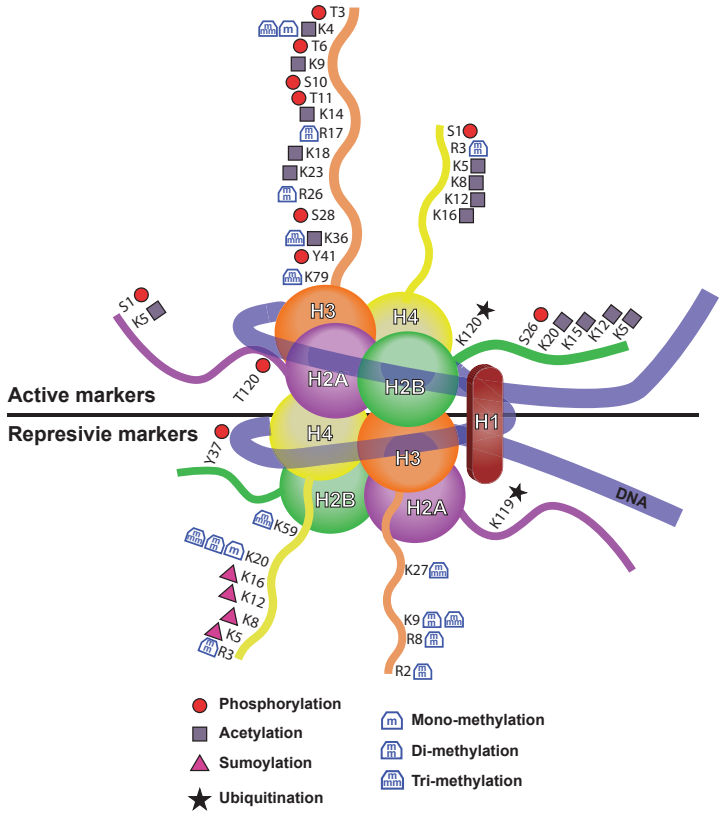


**Figure 4.** Schematic representation of the nucleosome. Two molecules of each of the four core histone proteins form the histone octamer, containing one tetramer of histone H3 and histone H4 and two dimers of histone H2A and H2B. DNA wrapped around a histone octamer forms the nucleosome. Histone H1 acts as a linker protein and holds the structure together. PTMs with known functions in gene expression are shown with the corresponding amino acids located on the histone tails (according to (Di Lorenzo and Bedford, 2011; Rossetto *et al.*, 2012; Shiio and Eisenman, 2003; Zhang *et al.*, 2015)). Active marks are represented in the upper part of the figure, and repressive marks are represented in the lower part of the figure. Lysine (K), arginine (R), serine (S), and threonine (T). For simplicity N- and C-terminal tails of H2A and H2B are represented as a single histone tail.

closed state (Bannister *et al.*, 2001; Lachner *et al.*, 2001). Also, instead of promoting protein binding to histones, histone methylation can disrupt an interaction between the histones and chromatin factors. For instance, tri-methylation of H3K4 prevents binding of nucleosome remodeling and deacetylase protein NuRD, thereby supporting transcription (Nishioka *et al.*, 2002; Zegerman *et al.*, 2002).

Phosphorylation of histones is another modification which plays a crucial role in chromatin dynamics (**Figure 4**). Histone phosphorylation is mainly known from γH2AX phosphorylation during DNA-damage, but there is a series of evidence implicating histone phosphorylation also in transcriptional regulation (reviewed in Rossetto *et al.*, 2012). Phosphorylation has been shown to crosstalk with other histone PTMs. For example, phosphorylation of H3 tail promotes H3 acetylation in a Gcn5-dependent manner (Lo *et al.*, 2000; Zhong *et al.*, 2003). Furthermore, phosphorylation of H3T6 was shown to prevent LSD1-mediated removal of H3K4 methylation, a mark of actively transcribed chromatin (Metzger *et al.*, 2010). Phosphorylation of H3T6 and H3T11 promotes removal of the repressive methyl mark on H3K9 by JMJD2C (Metzger *et al.*, 2008; Metzger *et al.*, 2010). H3S28 phosphorylation promotes demethylation of nearby K27 and promotes its acetylation (Lau and Cheung, 2011). In contrast, H2BY37 phosphorylation has been shown to repress transcription *via* preventing binding of transcriptional activator NPAT and subsequently decreasing Pol II recruitment (Mahajan *et al.*, 2012). In addition, H2BY37 phosphorylation facilitates recruitment of transcriptional repressors histone regulatory homolog A (HIRA) that enforce transcriptional silencing (Mahajan *et al.*, 2012).

Histones can also be ubiquitinated and/or sumoylated (**Figure 4**). Sumoylation acts as a transcriptional repressor, presumably through competing with histone acetylation (Shiio and Eisenman, 2003). While the function of histone mono-ubiquitination remains largely unknown, it has been shown that mono-ubiquitination of H2AK119 is involved in gene silencing (Wang *et al.*, 2004) and H2BK120 mono-ubiquitination plays a role in transcriptional initiation and elongation (Kim *et al.*, 2009; Lee *et al.*, 2007).

What is the role that chromatin play in regulation of the HSR? Studies using *HSP70 loci* in *Drosophila* showed that heat stress results in rapid poly(ADP-ribose) polymerase 1 (PARP1)-mediated clearance of nucleosomes from the gene body (Petesch and Lis, 2012). Surprisingly, loss of nucleosomes from *HSP70 loci* has also been achieved by chemical activation of HSF1 DNA-binding activity, without activating transcription (Petesch and Lis, 2008). Furthermore, global analyses of stress-inducible HSF1 target genes revealed that they contain distinctive chromatin marks which suggest that the local chromatin landscape pre-conditions the stress-inducible gene expression (Guertin and Lis, 2010; Mahat *et al.*, 2016; Vihervaara *et al.*, 2013). HSF1-binding sites are located in predominantly open chromatin regions as shown by nucleosome occupancy assays (Shivaswamy and Iyer, 2008; Wu, 1980). Moreover, there is a strong correlation between HSEs and active chromatin marks in unstressed conditions, indicating that HSF1-binding sites reside in an active chromatin (Guertin and Lis, 2010). The HSR has also been shown to undergo repression *via* specific chromatin

conformation. In *C. elegans*, the HSR, along with other stress responses, such as UPR and the Oxidative Stress Response, diminish with the progression of worm into adulthood (Labbadia and Morimoto, 2015). Repression of the HSR and other responses occurs due to an increase in tri-methylation of H3K27, which is a marker for repressed chromatin, at stress gene *loci*. This prevents HSF1 binding to its target genes, despite the protein being DNA-binding competent. The increase in the H3 methylation is caused by reduced expression of KDM jmjd-3.1 (Labbadia and Morimoto, 2015). In conclusion, both chromatin landscape and various transcriptional activators and repressors contribute to the activation and maintenance of the HSR.

### 2.4. Heat shock proteins

Heat shock protein (HSPs) are the main products of the HSR. They are the first and last line of defense against proteotoxic stresses, due to their ability to enforce correct folding, thereby restoring proper protein function (reviewed in Hartl *et al*., 2011). If the damage to proteome is too severe, HSPs guide damaged proteins to degradation and thus prevent the formation of toxic protein aggregates. Furthermore, HSPs due to their antiapoptotic abilities, allow cells to survive and recover from various proteotoxic conditions. HSPs are classified into families based on their molecular size and domain homology (Kampinga *et al*., 2009). In mammals, HSPA1A/B (HSP70), HSPB1 (HSP25), HSPC1 (HSP90), HSPH1 (HSP110) and DNAJB1 (HSP40) have been shown to be the main stress-induced HSPs. A detailed analysis of human HSF1 target genes revealed that upon acute heat stress HSF1 occupies 70% of HSPs, 90% of chaperonins and 13% of DNAJ genes (Vihervaara *et al.*, 2013). However, HSF1 is dispensable for HSPs expression under basal conditions (Mahat *et al*., 2016)

HSP70, HSP90, and HSP110 typically recognize hydrophobic amino-acid side chains exposed by non-native proteins and promote their folding through ATP- and cofactor-regulated binding and release cycles. Other HSPs, such as small HSPB family and type I and II chaperonins (HSPD1, HSPE1, and CCT family) work in an ATP-independent manner by binding to non-native proteins and holding them until they are refolded by HSP70/HSP90 machinery or cleared for proteasomal degradation. HSP70 assists proteins as early as they emerge from the ribosome, by binding to hydrophobic residues of nascent proteins (Hundley *et al*., 2002). The ATP-dependent reaction cycle of HSP70 is regulated by DnaJ family and nucleotide-exchange factors (NEFs) (reviewed in Kampinga and Craig 2010 and Mayer, 2010). NEFs are required for the recycling of hydrolyzed ADP that allows HSP70 opening, substrate release, and binding of a new client protein. DnaJ provides substrate specificity and increases the portfolio of HSP70 client proteins. Also, ATP-to-ADP hydrolysis is catalyzed by many of the DnaJ family members which allows for efficient folding of proteins.

HSP90 is one of the most abundant proteins in the cell. It works downstream of HSP70 facilitating structural maturation and conformational regulation of many signal-transduction molecules, such as protein kinases and steroid receptors. HSP90 binds to 7% of transcription factors, 30% of ubiquitin ligases and 60% of kinases in human cells

(Taipale *et al.*, 2012). Due to that, HSP90 serves as a hub affecting multiple cellular processes and is a key mediator of cellular homeostasis (McClellan *et al.*, 2007). HSP90 functions as a dimer that is assembled by the interaction of individual HSP90 C-terminal domains. ATP binding leads to the dimerization of the N-terminal domains, resulting in dimer condensation, where individual monomers twist. ATP hydrolysis results in separation of the N-terminal domains. During its opening-closing cycle, HSP90 cooperates with several regulators and co-chaperones (reviewed in Hartl *et al.*, 2011).

HSPs are present in every cellular compartment and are even secreted into the extracellular environment (reviewed in De Maio and Vazquez, 2013). HSPs and their co-chaperones form a complex network of molecular interactions, which act as a hub that guarantees cellular homeostasis (Taipale *et al.*, 2014). Thus, it is not surprising that HSPs have been found to play an important function in multiple human pathologies. For instance, many HSPs are found to be overexpressed in various cancers (reviewed in Lianos *et al.*, 2015). Cancer cells extensively rewire their metabolic and signaling pathways, which changes the intracellular environment and transformed cells become dependent on stress-inducible HSPs to maintain protein function. Furthermore, HSPs can directly promote oncogenesis thanks to their endogenous anti-apoptotic abilities.

HSPs are also perceived as a promising therapeutic target in neurodegenerative disorders (reviewed in Kampinga and Bergink, 2016). Due to their intrinsic ability to bind and stabilize misfolded proteins, increased HSP number and activity would prevent the formation of protein aggregates and amyloidogenic precursors. If the formation of protein aggregates have already occurred, HSPs have been shown to facilitate the moving of aggregates to specialized cellular compartments, where their toxicity is reduced (reviewed in Miller *et al.*, 2015). For many years it was believed that mammalian HSPs do not exhibit disaggregation ability, in contrast to *S. cerevisiae* HSP104 (Glover and Lindquist, 1998). However, recent studies provide evidence that the HSP70–J-protein–HSP110 chaperone network is able to efficiently clear existing cellular aggregates (Nillegoda *et al.*, 2015; Shorter, 2011).

HSPs are also able to ameliorate the adverse effects in other protein folding maladies. HSPs have been shown to have a cytoprotective function in various neurological stresses, such as ischemia, hemorrhage, and spinal cord injury (reviewed in Turturici *et al.*, 2011). Additionally, increased HSP70 levels have been shown to increase lifespan and slow down muscle failure in Duchenne muscular dystrophy mouse models (Gehrig *et al.*, 2012). Taken together the role of HSPs in various pathologies, there is a great need for pharmacological interventions to regulate HSPs expression.

### 2.5. Sensory mechanisms of HSR

The HSR can be activated by multiple forms of proteotoxic stress. However, our understanding of exact molecular pathways leading to HSF1-mediated HSPs expression is limited. To date, three non-exclusive mechanisms driving the HSR have been proposed. Several studies have shown that purified HSF1 protein is capable of

sensing protein-damaging conditions, such as elevated temperature, low pH, or ROS (**Figure 5A**) (Goodson and Sarge, 1995; Hentze *et al.*, 2016; Mosser *et al.*, 1988; Zhong *et al.*, 1998). When exposed to stress, HSF1 spontaneously forms trimers and gains DNA-binding activity. A recent study showed that transition of HSF1 to the DNA-binding state positively correlates with its concentration (Hentze *et al.*, 2016). This provides an explanation for the earlier observed spontaneous activation of overexpressed HSF1 (Sarge *et al.*, 1993). Furthermore, intrinsically activated HSF1 cannot reverse to the monomeric state, which imposes a requirement of additional factors in switching off the HSR (Hentze *et al.*, 2016).

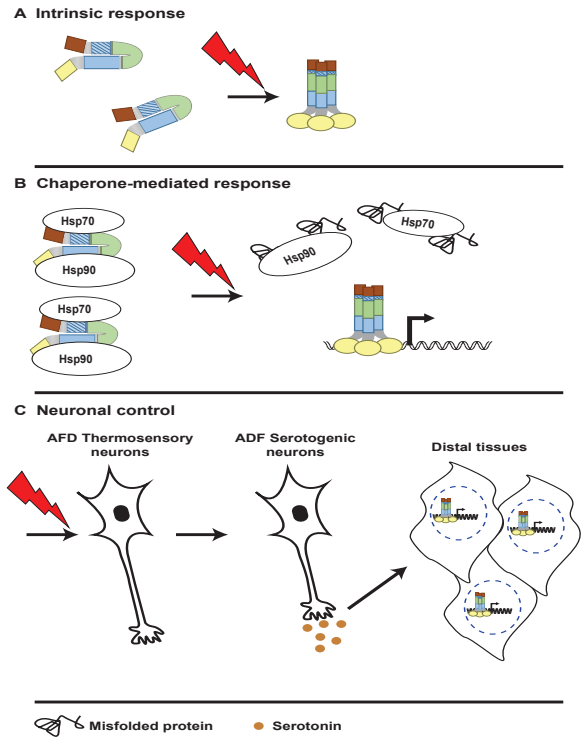


**Figure 5. Sensory mechanisms of the HSR. A)** Purified HSF1 upon exposure to elevated temperatures undergoes a monomer-to-trimer transition and gains DNA-binding capacity, which indicates an intrinsic mechanism of sensing heat stress. **B**) In the classical chaperone-mediated regulation of the HSR, it is proposed that under control conditions HSF1 interacts with molecular chaperones, especially HSP70 and HSP90. HSF1-HSP interaction keeps HSF1 inactive. Proteotoxic stress increases the amount of misfolded proteins in the cell, which titrates HSPs away allowing for HSF1 activation. **C)** In *C. elegans* exposed to heat stress, the thermosensory neurons AFD are activated, which leads to activation of the serotogenic neurons ADF. Serotonin release by ADF neurons allows for HSF1 activation in distal tissues, such as gonads.

The classical model of the HSR regulation assumes chaperone-mediated control of HSF1 activity (**Figure 5B**) (Hartl *et al.*, 2011). Under normal conditions, HSF1 trimerization, gain of DNA-binding activity and transcriptional competence are prevented by interaction with HSPs (Abravaya *et al.*, 1992; Neef *et al.*, 2014; Zheng *et al.*, 2016; Zou *et al.*, 1998). Protein-damaging stresses increase the amount of misfolded proteins, which titrates HSPs away from HSF1. That, in turn, allows for HSF1 trimerization and initiation of the HSR. HSP70 and HSP90 are postulated to be the main chaperones repressing the HSR under normal conditions. For instance, the depletion of either HSP70 or HSP90 in *C. elegans* leads to HSR activation (Guisbert *et al.*, 2013). *S. cerevisiae* appears to be an exception among studied species as only HSP70 and DnaJs are required for regulation of HSF1 activity (Zheng *et al.*, 2016). However, in another fungus, *C. albicans*, depletion of HSP90 leads to increase of HSF1 binding

already under normal conditions (Leach *et al.*, 2016). Furthermore, in human cells, pharmacological inhibition of HSP90 leads to activation of the HSR (reviewed in Neckers and Workman, 2012), which strongly suggests the involvement of HSP90 in the chaperone-mediated HSR regulation.

In multi-cellular organisms, an additional neuronal-mediated layer of HSF regulation has been reported (**Figure 5C**). In *C. elegans* activation of the HSR by elevated temperature can be disabled by introducing amorphic mutations into the AFD thermosensory neurons (Prahlad *et al.*, 2008). Intriguingly, the same mutation of AFD neurons did not have an effect on the HSR induced by heavy metals, indicating the existence of parallel mechanisms controlling the HSR in *C. elegans*. The physiological function of AFD neurons is to control the thermotactic behavior, which allows organisms to find a place with an optimal temperature for growth and reproduction (Clark *et al.*, 2007). A further study showed that inhibition of serotonin signaling markedly reduces the HSR, which highlights the importance of ADF serotogenic neurons and serotonin release in neuronal control of the HSR (Tatum *et al.*, 2015). However, there is no evidence for similar neuronal control of the HSR to exist in other metazoans.

**2.6. Inducers, co-inducers, and repressors of HSR**

Multiple studies have been performed in the search for compounds capable of inducing the HSR in an HSF1-dependent manner (reviewed in Dayalan Naidu and Dinkova-Kostova, 2017). The HSR activators can be categorized into two main groups (West *et al.*, 2012). Group A contains compounds which have known targets in the protein homeostasis networks. To this group belong the HSR activators, such as amino acid analogs that prevent protein from reaching its normal folding state (*e.g.*, azetidine 2-carboxylate, canavanine), various pharmacological inhibitors of molecular chaperones (*e.g.*, geldanamycin and radicicol), and proteasome and protease inhibitors (*e.g.*, MG132). Activators in Group B do not have clear targets, but they display general ability to induce protein damage, often on the cysteine residue, in an electrophilic manner. Examples of such activators are phytochemicals celastrol, curcumin, sulforaphane, and withaferin A, as well as synthetic compounds to which belong benzyl pyrazole derivatives. However, most of the currently used pharmacological activators of the HSR have cytotoxic effects, due to their protein-damaging properties severely hampering the drug development.

Another group of the HSR modulators are compounds which alone do not induce the HSR, but potentiate expression of HSPs in the presence of HSF1 activating stimuli, *i.e.* heat stress. To this group of the so-called chaperone co-inducers belongs chemicals, such as hydroxylamine derivative bimoclomol and arimoclomol (Vígh *et al.*, 1997), BGP-15 (Gombos *et al.*, 2011), resveratrol (Westerheide *et al.*, 2009), and phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (Holmberg *et al.*, 1997). Hydroxylamine derivatives and resveratrol have been shown to co-induce the HSR through delaying HSF1 attenuation from its target gene promoters (Hargitai *et al.*, 2003; Westerheide *et*

*al.*, 2009). Molecular mechanisms of action of BGP-15 and TPA remain largely unknown. It has been shown that TPA, which is a protein kinase C (PKC) activator, is capable of enhancing HSPs expression, despite inducing accelerated attenuation of HSF1 DNA-binding activity (Holmberg *et al.*, 1997). Although not being able to activate the HSR alone, chaperone co-inducers display remarkable therapeutic potential in various diseases models. Treatment with bimoclomol results in cytoprotective effect in models of diabetes mellitus, cardiovascular disease, and renal failure (reviewed in Nánási and Jednákovits, 2001). In addition, arimoclomol delays disease progression in amyotrophic lateral sclerosis mouse models (Kieran *et al.*, 2004). Resveratrol which is a well-known activator of SIRT1, a class III HDAC, has been shown to have beneficial effects in heart disease and brain disorders, however, this effect is not solely HSF1-dependent (reviewed in Dong *et al.*, 2016; and Petrovski *et al.*, 2011). Also BGP-15 has been shown to be advantageous in multiple disease models, such as insulin resistance (Henstridge *et al.*, 2014; Literáti-Nagy *et al.*, 2012), atrial fibrillation (Sapra *et al.*, 2014), muscle dystrophy (Gehrig *et al.*, 2012), and ventilation-induced diaphragm dysfunction (VIDD) (Salah *et al.*, 2016). Molecular mechanisms behind BGP-15 co-inducing capacity were under investigation in Study II of this thesis.

Activation of the HSR and increase in HSPs levels are not always beneficial. Cancer cells rely on HSPs activity to survive (reviewed in Lianos *et al.*, 2015). Hence, the discovery of small molecules inhibiting HSF1-mediated expression of HSPs is actively being pursued (Whitesell and Lindquist, 2009). Although the HSR inhibitors alone are potent anti-cancer drugs, they greatly increase the effect of therapy with some anti-cancer treatments. For instance, the HSF1 inhibitor quercetin increases the sensitivity of neuroblastoma cells to doxorubicin treatment (Zanini *et al.*, 2007) and increases radiofrequency ablation-induced destruction of tumors in rats (Yang *et al.*, 2016).

Treatment of cells with protein translation inhibitors, such as rocaglamide A, cephaeline and emetine reduce HSF1-mediated gene expression (Dai *et al.*, 2015; Santagata *et al.*, 2013). These results show that a feedback loop exists between protein translation, cellular metabolism and HSF1-mediated HSPs expression (Santagata *et al.*, 2013). A subsequent study revealed that metabolic stress causes HSR inhibition *via* metabolic stress sensor AMP-activated protein kinase (AMPK)-mediated HSF1 inactivation (Dai *et al.*, 2015). Dai and co-workers showed that AMPK activation with metformin, which is the most frequently prescribed medicine for type II diabetes worldwide (Thomas and Gregg, 2017), inactivates HSF1 DNA-binding ability, reduces HSPs expression and increases protein aggregation in cancer cells, which results in reduced tumor incidence and growth in a xenograft mouse model.

## 3. Heat Shock Factors

### 3.1. Family of heat shock factors

Identification of HSEs as promoter elements regulating stress-inducible HSPs expression allowed for the identification of the HSFs, as a driver of HSPs expression.

In *D. melanogaster* and *C. cerevisiae,* only a single HSF protein has been identified (Wiederrecht *et al.*, 1987; Wu *et al.*, 1987). However, in 1991 identification of two separate yet related HSE-binding proteins from human cells led to a conclusion that vertebrates express more than a single HSF (Rabindran *et al.*, 1991; Schuetz *et al.*, 1991).

To date, six HSFs have been found in mammals: HSF1, HSF2, HSF3, HSF4, HSFY, and HSFX (reviewed in Åkerfelt *et al.*, 2010). In plants, more than thirty HSFs have been identified (reviewed in Scharf *et al.*, 2012). Limited information is available on mammalian HSF3, so far found only in mice (Fujimoto *et al.*, 2010), and HSFY and HSFX, which are sex chromosome-specific transcription factors (Bhowmick *et al.*, 2006; Kinoshita *et al.*, 2006; Shinka *et al.*, 2004).

HSF1 is a master regulator of the HSR in vertebrates, and its function and regulation will be discussed in more detail in Chapter 3.3. HSF2 shares a ~70% identity of the amino acid sequence in the DNA-binding domain (DBD) and oligomerization (HR-A/B) domain, and ~35% identity in the rest of the protein with HSF1 (Pirkkala *et al.*, 2001). HSF2 is expressed in a wide range of mammalian tissues, yet its expression levels greatly vary from tissue to tissue (Fiorenza *et al.*, 1995). HSF2 is important for normal development and proper gametogenesis. Studies using *hsf2-null* mice showed defective neuronal migration indicating that HSF2 plays an important role in the development of the cerebral cortex (Chang *et al.*, 2006). In developing neurons, HSF2 drives regulation of genes, such as p35, p39, T-box brain gene 1 (Tbr1), and mitogen-activated proteins (MAPs) which are essential for proper neuronal migration (Chang *et al.*, 2006; El Fatimy *et al.*, 2014; Wang *et al.*, 2003b). Interestingly, HSF2 is detrimental in the developing cortex chronically exposed to alcohol (El Fatimy *et al.*, 2014). Alcohol exposure of developing brain leads to the constitutive activation of HSF1 and formation of transcriptionally deficient HSF1-HSF2 heterotrimers. These results in the decreased expression of MAP genes and promotes defects characteristic for Fetal Alcohol Syndrome (FAS).

HSF2 deficiency results in decreased male and female fertility (reviewed in Abane and Mezger, 2010). In male mice, HSF2 is most abundantly expressed in testis, and its levels vary during different stages of spermatogenesis (Alastalo *et al.*, 1998; Fiorenza *et al.*, 1995). In developing spermatocytes, HSF2 was found to occupy multiple genomic *loci*, and in addition to HSPs, HSF2 regulates expression of genes that are specifically important for sperm quality (Åkerfelt *et al.*, 2008; Korfanty *et al.*, 2014). Examples of these genes are Y-chromosomal multi-copy genes, *e.g.* spermiogenesis specific transcript on the Y 2 (Ssty2), Sycp3-like Y-linked (Sly), and Sycp3-like X-linked (Slx) (Åkerfelt *et al.*, 2008). HSF2 knockout in female mice reduced numbers of ovulated oocytes, and 70% of the fertilized oocytes appeared to be abnormal (Kallio *et al.*, 2002; Wang *et al.*, 2003b). However, the molecular events affecting female fertility in *hsf2-null* mice are not entirely elucidated.

In 2002, it was reported that mutations in the HSF4 DNA-binding domain are associated with the development of cataract in humans (Bu *et al.*, 2002). Indeed, the

loss-of-function mutation or deletion of HSF4 results in abnormal lens development in post-natal mice, which leads to cataract. In the lens tissue, HSF4 drives expression of γ-crystallin, which is the major structural protein in the lens (Fujimoto *et al.*, 2004; Shi *et al.*, 2009) Furthermore, HSF4 contributes to normal lens development, by interacting, stabilizing and activating the cell cycle suppressor protein p53 (Huang *et al.*, 2015). Despite that HSF4 is expressed in other tissues than lens, cataract patients with HSF4 mutations have no other symptoms, suggesting that HSF4 function is critical only in the lens. Specific mutations in the HSF4 protein have also been associated with age-related cataract, indicating that HSF4 function is required not only for the development of the lens but also for maintaining its homeostasis during aging (Jing *et al.*, 2014; Shi *et al.*, 2008).

### 3.2. The intramolecular organization of heat shock factors

The classification of proteins into the HSF family is based on the high amino acid sequence homology of the DBD which belongs to the winged helix-turn-helix family and is shared by all HSFs (**Figure 6**) (Damberger *et al.*, 1994; Harrison *et al.*, 1994; Jaeger *et al.*, 2016; Neudegger *et al.*, 2016; Vuister *et al.*, 1994). Trimerization of HSFs is facilitated by another functional domain that is structurally conserved throughout evolution, the oligomerization domain (HR-A/B), composed of leucine zipper-like heptad repeats (Sarge *et al.*, 1993; Westwood and Wu, 1993). Intact HR-A/B is required for formation of HSF trimers that are capable of binding to DNA (Jaeger *et al.*, 2016; Sarge *et al.*, 1993). Other HSFs domains, such as the regulatory domain (RD) and activatory domain (AD), greatly vary in size and amino acid composition and are thus defined based on their functions.

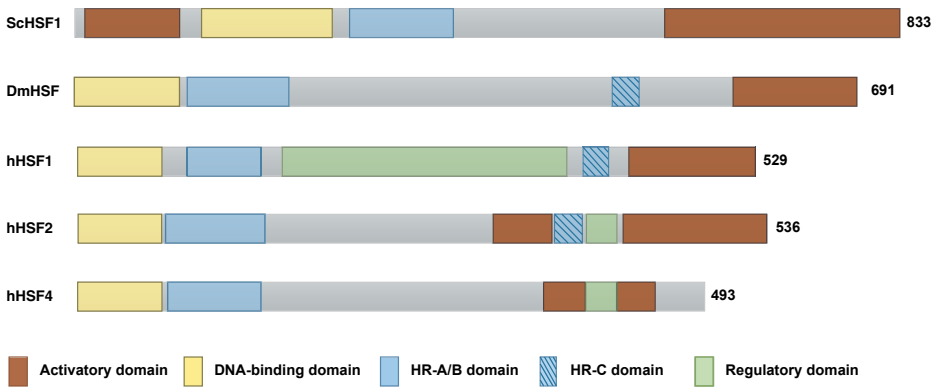


**Figure 6. HSFs are composed of similar functional domains.** Schematic illustration of *S. cerevisiae* HSF1 (ScHSF1), *D. melanogaster* (DmHSF1), human HSF1 (hHSF1), human HSF2 (hHSF2), and human HSF4 (hHSF4). HR-A/B/C – leucine-zipper-like heptad repeat domains. The last amino acid of each protein is indicated. The figure is not drawn to scale.

The DBD of HSFs recognizes and binds to the regulatory sequence known as the heat shock element (HSE), consisting of inverted pentameric nGAAn repeats (Amin *et al.*, 1988). The minimal HSE recognized by HSFs has at least three continuous inverted repeats of nGAAn (Amin *et al.*, 1994; Fernandes *et al.*, 1994; Perisic *et al.*, 1989).

Although the HSE architecture facilitating HSF binding can vary in sequence, spacing, orientation, and number of repeats (Enoki and Sakurai, 2011; Fernandes *et al.*, 1994; Jaeger *et al.*, 2014), the genome-wide analyses of HSF binding motifs across metazoans revealed a striking similarity in the core HSE sequence (Guertin and Lis, 2010; Li *et al.*, 2016; Mendillo *et al.*, 2012; Vihervaara *et al.*, 2013).

The crystal structures of yeast HSF DBD bound to HSE were obtained already in the 90's and showed that unlike many other winged helix-turn-helix proteins, the HSF wing domain does not contact DNA (Littlefield and Nelson, 1999). Two recent structural studies revealed that HSF proteins embrace DNA, by positioning their DBDs and HR-A/B domains on the opposite sites of the DNA strand (Jaeger *et al.*, 2016; Neudegger *et al.*, 2016). This unique conformation results in stable HSF binding to DNA and exposes the surfaces of wing domains as well as the DBDs for different PTMs and other regulatory inputs.

The trimerization of HSFs occurs *via* intermolecular interactions between the HR-A/B domains of adjacent HSF monomers (Peteranderl *et al.*, 1999). The spontaneous trimerization of HSFs under normal conditions is suppressed by another heptad repeat domain, called HR-C. Under non-stressed conditions, HR-C interacts with HR-A/B, maintaining HSF in a monomeric state (Hentze *et al.*, 2016). HSFs which do not contain HR-C domain, such as *S. cerevisiae* HSF1 (ScHSF1) and HSF4 are found in the constitutive DNA-bound state (Jakobsen and Pelham, 1991; Nakai *et al.*, 1997). The AD that is required for the *trans*-activating capacity of HSFs is found in their C-terminal regions, except for ScHSF1 that harbors an activatory domain both in the N- and C-terminus (**Figure 6**) (Nieto-Sotelo *et al.*, 1990; Shi *et al.*, 1995; Yoshima *et al.*, 1998). HSF4 for many years was thought to lack an AD, but a recent study identified an AD in the C-terminus (Merath *et al.*, 2013). The AD is largely unfolded, enriched in hydrophobic and acidic amino acid residues (Green *et al.*, 1995; Newton *et al.*, 1996; Shi *et al.*, 1995), which has been shown to facilitate interactions with other transcription factors and co-factors and ensure rapid and accurate activation to stress stimuli (Boellmann *et al.*, 2004; Park *et al.*, 2001; Sullivan *et al.*, 2001). The RD represses AD under normal conditions, senses stress, and allows stress-inducible activation (Green *et al.*, 1995; Newton *et al.*, 1996). Among HSFs the mammalian HSF1 RD is most studied (Green *et al.*, 1995; Hietakangas *et al.*, 2006; Knauf *et al.*, 1996; Newton *et al.*, 1996), but the corresponding regions have also been found in HSF2 and HSF4 (Merath *et al.*, 2013; Yoshima *et al.*, 1998; Zhu and Mivechi, 1999).

### 3.3. Heat shock factor 1

HSF1 regulates the HSR in all metazoans. HSF1 is the best-characterized member of HSF1 family. HSF1 is constitutively expressed in most tissues and cell types and appears to be controlled primarily through PTMs (Anckar and Sistonen, 2011). Although initially discovered as the regulator of the stress-induced expression of HSPs, today HSF1 is perceived as an important physiological and pathological factor. HSF1 is required for healthy development and gametogenesis. Transcriptional

programs controlled by HSF1 position HSF1 as a promising target for therapeutic strategies in cancer and proteinopathies.

### 3.3.1. Post-translational regulation of HSF1

In cells, HSF1 undergoes numerous PTMs, such as acetylation, phosphorylation, sumoylation, and ubiquitination, which regulate HSF1 activity (**Figure 7**) (reviewed in Xu *et al.*, 2012). Phosphorylation is a hallmark modification of HSF1, and it occurs on multiple sites in *S. cerevisiae*, *D. melanogaster*, and mammalian HSF1 (Fritsch and Wu, 1999; Guettouche *et al.*, 2005; Zheng *et al.*, 2016). To date, 23 phosphorylation sites have been identified on serine and threonine residues in human HSF1. HSF1 can be phosphorylated both *in vitro* and *in vivo* by many kinases, including MAPKAPK2 (Guettouche *et al.*, 2005; Wang *et al.*, 2006), CK2 (Gomez-Pastor *et al.*, 2017; Soncin *et al.*, 2003), PLK1 (Lee *et al.*, 2008), CaMKII (Holmberg *et al.*, 2001), GSK3β (Chu *et al.*, 1996; Chu *et al.*, 1998), PKC (Chu *et al.*, 1998), PKA (Murshid *et al.*, 2010), and PLK1 (Kim *et al.*, 2005a). Moreover, a recent study showed that HSF1 is phosphorylated directly by MEK, which makes HSF1 a second MEK target, after ERK (Tang *et al.*, 2015).

Because a majority of the phosphorylation sites in HSF1 reside within the RD and HSF1 activation coincides with stress-inducible phosphorylation, it has been hypothesized that phosphorylation acts as a switch from transcriptionally inactive to the active form of HSF1 (Guettouche *et al.*, 2005; Knauf *et al.*, 1996). The requirement of phosphorylation for HSF1 activation has been addressed in Study I of this thesis.

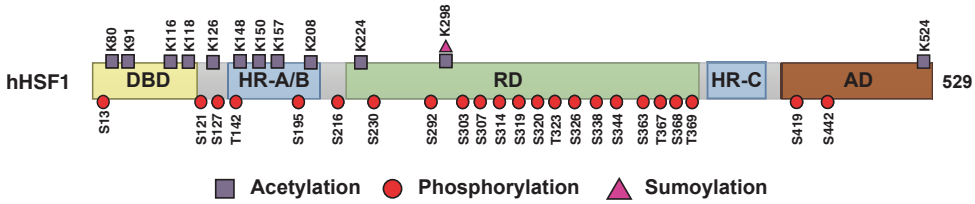


**Figure 7. Post-translational modifications of human HSF1.** hHSF1 is a heavily phosphorylated protein (23 known phosphorylation sites), with the majority of phosphorylation sites located in the regulatory domain (RD). Other domains of HSF1: DNA-binding domain (DBD), oligomerization domain (HR-A/B), C-terminal heptad repeat domain (HR-C) and activatory domain (AD). HSF1 undergoes acetylation mainly within DBD and HR-A/B. To date, K298 is the only experimentally validated sumoylation site on HSF1. HSF1 is also ubiquitinated, but the specific residues are unknown. The figure is not drawn to scale.

Phosphorylation adds a strong negative charge to the protein surface that creates an interface which mediates formation of protein-protein complexes (Nishi *et al.*, 2011). Furthermore, phosphorylation has been shown to change protein conformation (Johnson and Barford, 1993). Studies on functional consequences of HSF1 phosphorylation have revealed that phosphorylation has both stimulatory and inhibitory effect on HSF1 *trans*-activation capacity, depending on the target phosphorylation site. For instance, phosphorylation of S326 is widely recognized as a marker of HSF1 activity (Boellmann *et al.*, 2004; Mendillo *et al.*, 2012; Shah *et al.*, 2016). It has been shown that S326 can be phosphorylated by several kinases including MEK

(Tang *et al.*, 2015), mTOR (Chou *et al.*, 2012), and p38 MAPK (Dayalan Naidu *et al.*, 2016). However, it remains to be established how phosphorylation of S326 contributes to enhanced *trans*-activation capacity of HSF1. It has been suggested that phosphorylation of S326 would facilitate HSF1 interaction with transcriptional activator Daxx (Boellmann *et al.*, 2004), but there is no compelling evidence for or against that hypothesis. Phosphorylation of S320 by PKA has been shown to direct HSF1 to the nucleus, which is required for efficient induction of HSPs in the event of stress (Murshid *et al.*, 2010). Another phosphorylation site that increases inducible expression of HSPs is S230. CaMKII phosphorylates S230, and both mutation of S230 and pharmacological inhibition of CaMKII reduce the HSR (Holmberg *et al.*, 2001), but again the precise mechanism is unknown. It is also plausible that phosphorylation of HSF1 RD changes its conformation, which enhances HSF1 *trans*-activation capacity. This hypothesis is supported by the notion that HSF1 RD is largely an unstructured domain (Hentze *et al.*, 2016), in which phosphorylation might induce disorder-to-order transition (Bah *et al.*, 2014).

Another group of phosphorylation sites are those which negatively impact HSF1 *trans*-activation capacity. Phosphorylation of S121 by AMPK prevents HSF1 DNA-binding, which significantly reduces HSF1 transcriptional activity (Dai *et al.*, 2015). S303 and 307 phosphorylation by GSK3β and ERK, respectively, has been shown to facilitate 14-3-3ε-mediated nuclear exclusion of HSF1 (Wang *et al.*, 2003c), however these results were contradicted by another study, where mutation of S303 and S307 did not have any effect on HSF1 localisation (Hietakangas *et al.*, 2003). In addition, phosphorylation of S303 and S307 facilitates binding of ubiquitin E3 ligase FBXW7$\alpha$, which leads to HSF1 degradation (Gomez-Pastor *et al.*, 2017). Interestingly, a cross-talk between kinases phosphorylating S307 and S326 was recently reported, showing that S326 phosphorylation by MEK represses S307 phosphorylation and that ERK impacts HSF1 S326 and S307 phosphorylation *via* MEK inhibition (Tang *et al.*, 2015). These results in the feedback loop between PTMs mediating transcriptional activation and repression of HSF1.

HSF1 is sumoylated on a lysine located within a specific motif, ΨKxExxSP named the phosphorylation-dependent sumoylation motif (PDSM), where Ψ denotes a branched hydrophobic amino acid (usually a leucine, isoleucine, or valine) (Hietakangas *et al.*, 2006). Within the PDSM, phosphorylation of S303 is required for sumoylation to occur at K298 (Hietakangas *et al.*, 2003). It is proposed that phosphorylation of S303 and subsequent K298 sumoylation of HSF1 would restrict its *trans*-activating capacity, but the exact mechanism remains unknown (Hietakangas *et al.*, 2006). To date, K298 is the only experimentally validated HSF1 sumoylation site. However, a recent mass-spectrometry study revealed that HSF1 undergoes sumoylation on 14 additional amino acids, which are located mainly within DBD and HR-A/B (Hendriks *et al.*, 2017), which suggests that sumoylation is involved in the regulation of HSF1 DNA-binding and trimer formation.

HSF1 is ubiquitinated, which results in proteasome-mediated degradation of HSF1 (Gomez-Pastor *et al.*, 2017; Kim *et al.*, 2016; Raychaudhuri *et al.*, 2014), but specific ubiquitination sites have not been reported. Recently, however, Gomez-Pastor and co-workers (2017) showed that HSF1 ubiquitination in cells expressing aggregation-prone proteins is dependent on the phosphorylation status of S303 and S307, which are phosphorylated by kinase CK2'. Phosphorylation of S303 and S307 creates a surface that allows binding of FBXW7$\alpha$, a substrate-targeting subunit of the SCF (Skp1–Cul1–F box) ubiquitin E3 ligase complex, which catalyzes ubiquitination and degradation of HSF1 (Gomez-Pastor *et al.*, 2017). HSF1 is also subjected to acetylation (Raychaudhuri *et al.*, 2014; Westerheide *et al.*, 2009). Acetylation of HSF1 plays a dual role, as acetylation within the DBD, such as at K80 and K118, inhibits DNA binding during the attenuation phase (Westerheide *et al.*, 2009), whereas acetylation of K208 and K298, located within HR-A/B and RD, stabilizes HSF1 (Raychaudhuri *et al.*, 2014). Interestingly, K298 of HSF1 is a target for both acetylation and sumoylation (**Figure 7**). Since these modifications have distinct effects on HSF1 activity, it is tempting to speculate that they function as a molecular switch, regulating HSF1 activity. For instance, acetylation of lysines would prevent their ubiquitination, thereby enhancing HSF1 stability.

### 3.3.2. HSF1 activation-attenuation pathway

Under normal growth conditions, HSF1 is kept in an inactive state by intramolecular and intermolecular interactions (Hentze *et al.*, 2016; Shi *et al.*, 1998). HSF1 has a nuclear localization signal (NLS) which allows for its nuclear localization (Vujanac *et al.*, 2005), but in the absence of stress, HSF1 shuttles between the cytoplasm and nucleus. It was also shown that a small portion of HSF1 is trimerized and bound to the promoter regions of HSPs already under normal conditions (**Figure 8**) (Vihervaara *et al.*, 2013). This DNA-bound HSF1 interacts with replication protein A (RPA) that is involved in the replication and DNA repair (Fujimoto *et al.*, 2012). HSF1-RPA interaction recruits the histone chaperone FACT and a chromatin remodeling complex containing BRG1, which maintains the *HSP70* promoter in the nucleosome-free state (Fujimoto *et al.*, 2012). DmHSF1 interacts with the poly(ADP-ribose) polymerase 1 (PARP1), and PARP1 is responsible for nucleosome removal from the *HSP70 loci* upon stress stimuli (Petesch and Lis, 2012). Human HSF1 has also been found to interact with PARP1 (Ouararhni *et al.*, 2006), but it remains to be established, whether the mammalian PARP1 has a similar function to that detected in *Drosophila*.

Upon protein-damaging stress, HSF1 forms trimers, accumulates in the nucleus, and binds to the HSEs in the promoters and enhancers of its target genes (reviewed in Dayalan and Dinkova-Kostova, 2017). Active HSF1 recruits other transcriptional activators, such as the Mediator complex (Park *et al.*, 2001), activating transcription factor 1 (ATF1) (Takii *et al.*, 2015), SWI/SNF chromatin remodeling complex containing BRG1 (Corey *et al.*, 2003; Sullivan *et al.*, 2001; Takii *et al.*, 2010), histone methyltransferase mixed-lineage leukemia 1 (MLL1) (Chen *et al.*, 2014), death associated protein-6 (Daxx) (Boellmann *et al.*, 2004), and co-activator activating signal

co-integrator 2 (ASC-2) (Hong *et al.*, 2004). *In vitro* HSF1 is able to interact with the basal transcription component TAF-9 (Choi *et al.*, 2000), but it is unknown whether HSF1 directly recruits GTFs to the HSP promoters in cells. In overall, HSF1 binding to the HSPs genes initiates a cascade of events that leads to release of the paused Pol II into productive elongation and subsequent mRNA synthesis (**Figure 8**).

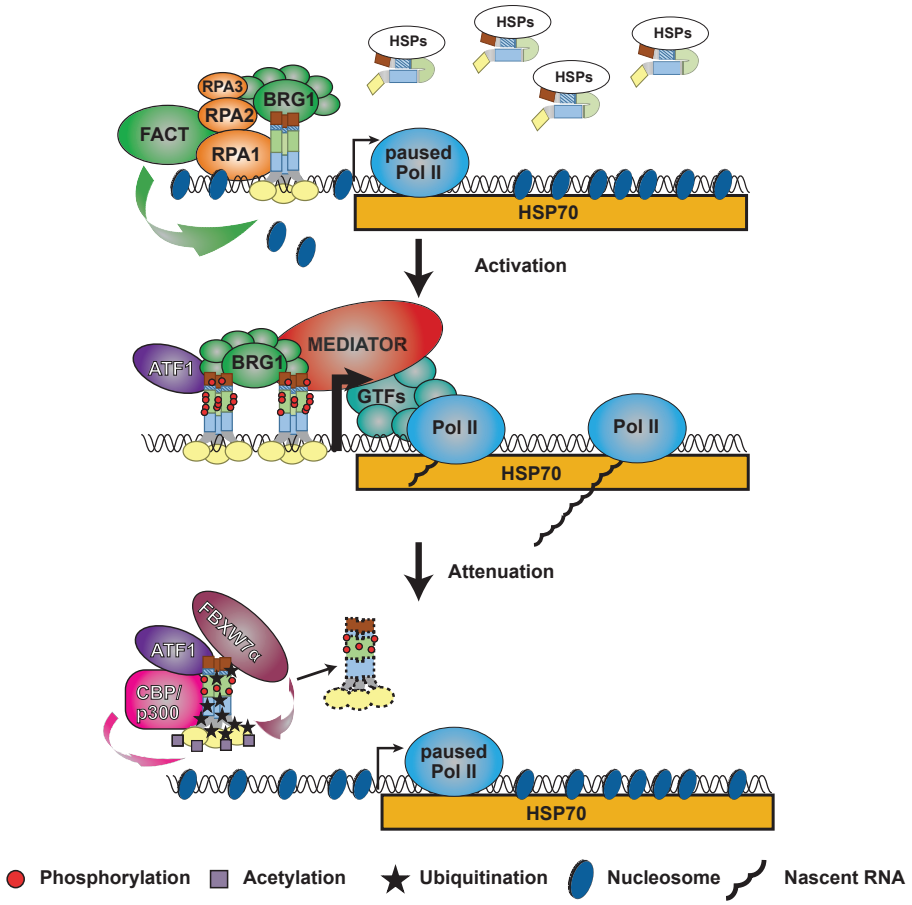


**Figure 8. HSF1 activation-attenuation pathway.** Under normal conditions, a majority of HSF1 is present in cells as inactive monomers interacting with HSPs. A small fraction of HSF1 is binding to the promoter of *HSP70*, where it interacts with replication protein A (RPA) and histone chaperone FACT. This interaction keeps the promoter region open. The *HSP70* gene is preloaded with the paused RNA polymerase II (Pol II). Upon activation, HSF1 trimerizes, accumulates in the nucleus and binds to DNA. The DNA-bound HSF1 interacts with other transcriptional regulators, such as general transcription factors (GTFs), activating transcription factor 1 (ATF1), transcription activator BRG1, and Mediator complex, which subsequently leads to release of the paused Pol II to elongation. Active HSF1 is phosphorylated, mainly within the regulatory domain, which fine-tunes the magnitude of the HSR. During the attenuation phase, HSF1 interacts with CBP/p300 in an ATF1-dependent manner. CBP/p300 acetylates the HSF1 DNA-binding domain, thereby preventing binding to DNA. Attenuation of HSF1 DNA-binding keeps Pol II in the paused state and stop transcription. Furthermore, HSF1 interacts with ubiquitin ligase FBXW7α, resulting in the degradation of nuclear HSF1 (dashed lines). Functional domains of HSF1 are color-coded as in Figures 6 and 7.

The exact molecular mechanisms initiating shutting down the HSF1 activity are not known. Negative feedback from newly produced HSPs was proposed as a mechanism forcing HSF1 attenuation. Indeed, in the course of stress HSP70 interacts with HSF1, thereby recruiting transcriptional co-repressor of RE1-silencing transcription factor (CoREST), which represses the HSF1-mediated transcription (Gómez *et al.*, 2008). Another mechanism that causes the release of HSF1 from its target promoters is acetylation of the HSF1 DBD which prevents HSF1 from binding to DNA (Westerheide *et al.*, 2009; Zelin and Freeman, 2015). The acetyltransferase complex CBP/p300 has been shown to acetylate HSF1 (Raychaudhuri *et al.*, 2014). Interestingly, ATF1 which enhances the *trans*-activating capacity of HSF1 during stress (Takii *et al.*, 2015), has an opposite effect during the attenuation phase, where it recruits CBP/p300 to HSF1. Acetylation of HSF1 is counteracted by histone deacetylases SIRT1, HDAC7, and HDAC9, thereby prolonging the HSR (Westerheide *et al.*, 2009; Zelin and Freeman, 2015).

Classical models postulated that inactivated HSF1 trimers dissociate back to monomers. However, recent findings suggest that attenuated HSF1 trimers mainly undergo proteasomal degradation (Kourtis *et al.*, 2015; Raychaudhuri *et al.*, 2014). Raychaudhri and co-workers reported that during recovery from heat shock, activated HSF1 did not revert to the less phosphorylated species but was degraded by the proteasome. In another study, Kourtis and co-workers reported that HSF1 interacts with FBXW7$\alpha$, which ubiquitinates HSF1 and promotes its degradation in the attenuation phase of the HSR (Kourtis *et al.*, 2015). Depletion of FBXW7$\alpha$ from cells led to the accumulation of nuclear HSF1 during recovery from proteotoxic stresses. Furthermore, loss of FBXW7$\alpha$ prolonged HSF1 binding to its target promoters and extended the HSR (Kourtis *et al.*, 2015). In addition, the observation that trimeric HSF1 is not capable of reverting to monomeric form *in vitro* (Hentze *et al.*, 2016), supports the model, where trimerized HSF1 would not dissociate to monomers, but instead is actively degraded during the attenuation phase (**Figure 8**).

### 3.3.3. Physiological role of HSF1

Studies of HSF1-null organisms have revealed that the HSF1 functions are not limited only to proteotoxic stress responses (**Figure 9**). The HSF1-transcriptional program is necessary for healthy development, gametogenesis, and maintaining protein homeostasis during aging of organisms. Furthermore, HSF1 has also been shown to indirectly regulate translation independently of its *trans*-activation capacity (Su *et al.*, 2016).

ScHSF1 is essential under normal growth conditions, and lack of ScHSF1 results in loss of cell wall integrity and arrests the cell cycle (Gallo *et al.*, 1993; Imazu and Sakurai, 2005; Sorger and Pelham, 1988). A recent study provided an explanation for the essential role of HSF1 in *S. cerevisiae*. Solís and co-workers showed that ScHSF1 under basal conditions regulates expression of 18 genes, encoding mainly molecular chaperones (Solís *et al.*, 2016). Surprisingly, the cell growth was rescued in ScHSF1-

depleted yeast by the constitutive expression of only two genes, *i.e.* HSP70 and HSP90. This demonstrates that the essential function of ScHSF is to drive expression of molecular chaperones that are necessary for maintaining protein homeostasis.


**Figure 9. Multiple functions of HSF1.** HSF1 is implicated in various physiological and pathological processes. HSF1 contributes to these processes as a transcriptional regulator, except protein translation.

In *C. elegans*, amorphic HSF1 mutations result in developmental arrest in the L1/L2 larval stage (Morton and Lamitina, 2013). During development, *C. elegans* HSF1 (CeHSF1) drives the expression of a specific set of genes required for proper larval development (Li *et al.*, 2016). CeHSF1 directly regulates genes involved in RNA biogenesis and translation, protein folding, processing, and transport, and metabolism. The function of CeHSF1 as a developmental factor is dependent on the unique promoter architecture containing a binding motif of transcription factor E2F that functions as a CeHSF1 co-activator (Li *et al.*, 2016). CeHSF1 has also been shown to regulate organisms longevity. In metazoans expression of anti-apoptotic genes is governed *via* the highly conserved insulin/insulin-like signaling (ILS) pathway (reviewed in Mathew *et al.*, 2017). Activation of the ILS pathway results in phosphorylation of transcription factor Daf-16/FOXO, which prevents Daf-16 nuclear accumulation and activation of genes that maintain youthfulness. Reduced ILS signaling in *C. elegans* increases organismal lifespan. However, the phenotype is reversed when either CeHSF1 or Daf-16 is downregulated (Hsu *et al.*, 2003; Morley and Morimoto, 2004). Similarly, overexpression of CeHSF1 prolongs lifespan, but only if Daf-16 is intact. Subsequent studies showed that activation of ILS signaling promotes the formation of DDL-1-containing HSF1 inhibitory complex (DHIC), which decreases the pool of available CeHSF1 (Chiang *et al.*, 2012). Reduction in the ILS signaling disrupts DHIC by enhanced DDL1 phosphorylation, which increases CeHSF1 activity under stress and in control conditions. It is, however, unknown if ILS signaling regulates HSF1 activity in vertebrates.

In mammals, HSF1 is an important factor in development and gametogenesis. *hsf1*$^{-/-}$ mice display several developmental defects, such as increased prenatal lethality, growth retardation, complete female infertility, abnormalities of the olfactory epithelium, and aberrant development of neurons (Kallio *et al.*, 2002; Uchida *et al.*, 2011; Xiao *et al.*, 1999). Fertilized oocytes originating from *hsf1*$^{-/-}$ mice are not able to develop past the zygotic stage, even when mice are mated with wild-type males, which indicates that HSF1 is a maternal factor (Benjamin *et al.*, 2000). A subsequent study revealed that HSF1 regulates expression of HSP90$\alpha$ in maturing oocytes, to promote proper meiotic maturation and asymmetrical division (Metchat *et al.*, 2009). These findings suggest that HSF1 through regulating HSP90, and possibly other HSPs, is essential for the reproductive success of pre-implantation embryos. HSF1 is required for proper myelin formation, neuron maturation, lateral ventricles and hippocampal development (Santos and Saraiva, 2004; Uchida *et al.*, 2011), but the exact mechanism of action is not entirely elucidated.

A recent study presented a mechanism by which HSF1 controls organismal growth (Su *et al.*, 2016). The mechanistic target of rapamycin complex 1 (mTORC1) is a prominent regulator of translation which also acts as a sensor for environmental stresses (reviewed in Laplante and Sabatini, 2012). c-JUN N-terminal kinase (JNK) interacts with mTORC1 that in turn inhibits protein translation (Su *et al.*, 2016). In cells exposed to stress, HSF1 directly interacts and suppresses JNK to promote mTORC1-mediated translation, which is critical for maintaining protein synthesis. *hsf1*$^{-/-}$ mice which suffer from growth retardation display an increased JNK but diminished mTORC1 activity. The HSF1&JNK double knock-out restored the cell, organ and body size, indicating that the HSF1-JNK-mTORC1 axis controls post-natal organismal growth (Su *et al.*, 2016). Surprisingly, HSF1 mutants that lack transcriptional capacity were able to suppress JNK activity and maintain mTORC1 activity (Su *et al.*, 2016). This is the first example of HSF1 functioning independently of its canonical transcriptional action.

### 3.3.4. HSF1 in cancer

In 2007, two articles reported an impact of HSF1 on tumorigenesis (Dai *et al.*, 2007; Min *et al.*, 2007). Dai and co-workers showed that mice lacking HSF1 develop fewer tumors when exposed to chemical skin carcinogenesis using a clinically relevant p53 mutation mouse model (Dai *et al.*, 2007). Similarly, Min and co-workers presented that in *p53*$^{-/-}$ mice, which typically develop lymphomas, the loss of HSF1 resulted in a marked reduction of lymphomas (Min *et al.*, 2007). Today, it is well established that HSF1 is an important player in tumorigenesis (reviewed in Dai and Sampson, 2016). For instance, HSF1 transcriptional activation is associated with the poor prognosis for breast cancer patients (Santagata *et al.*, 2011). Malignant cells carry multiple genetic and epigenetic alterations and are exposed to more stresses than healthy cells. Thus, originally it was proposed that HSF1 promote malignancy by increasing HSPs expression, to maintain protein homeostasis in cancer cells (Solimini *et al.*, 2007). However, it was found that HSF1 in cancer drives a transcriptional program different from that in stress responses,

and thus directly promotes tumorigenesis (Mendillo *et al.*, 2012). In addition to HSPs, HSF1 regulates transcription of genes involved in cell cycle regulation, chromatin remodeling, translation, signaling pathways, and metabolism (**Figure 10**). HSF1 binds to almost 900 genes in highly malignant breast cancer cells under control conditions (Mendillo *et al.*, 2012). Furthermore, loss of HSF1 in cancer cells increases expression of genes involved in immune functions, insulin secretion, and apoptosis, whereas expression of genes involved in protein folding, translation, and mitosis is decreased. This cancer-specific transcriptional program has been found in breast and colon cancer patient samples as well as in cell lines derived from breast, colon, and lung cancers (Mendillo *et al.*, 2012).

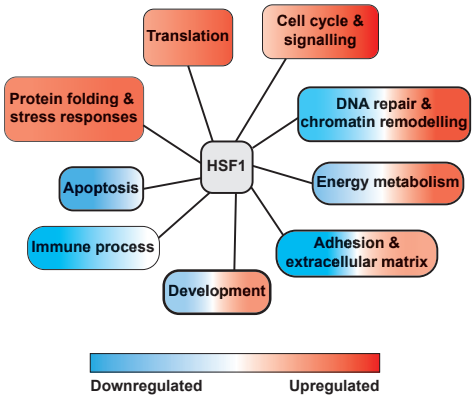


**Figure 10. HSF1 regulates a specific transcriptional program in cancer.** HSF1 cancer program differs from that of the HSR. In cancer cells, HSF1 upregulates genes involved in protein folding, translation, and cell cycle, while it downregulates genes involved in apoptosis and immune response. Genes contributing to energy metabolism, development, chromatin remodeling, and mRNA processing are both upregulated and downregulated by HSF1. Adapted from (Mendillo *et al.*, 2012).

Importantly, cancer cells have been shown to depend on HSF1 activation in the tumor stroma, to support their growth (Scherz-Shouval *et al.*, 2014). In cancer-associated fibroblasts representing the stroma, HSF1 drives a distinct transcriptional program, where it activates genes involved in angiogenesis, extracellular matrix (ECM) organization, adhesion, and migration (Scherz-Shouval *et al.*, 2014). Mechanisms which drive HSF1 activation in cancer remain to be elucidated. It has been proposed that cancer-specific alterations in signaling pathways, such as epidermal growth factor receptor HER2 (Zhao *et al.*, 2009) and the RAS-RAF-MEK signaling.(Stanhill *et al.*, 2006; Tang *et al.*, 2015) would activate HSF1. Zhao and co-workers (2009) have shown that overexpression of oncogene HER2 upregulates HSF1. HSF1, in turn, drives the expression of lactate dehydrogenase A supporting tumor glycolysis. While it has been reported that increased levels of HSF1 in cells facilitate activation of HSF1 in the absence of stress (Hentze *et al.*, 2016; Sarge *et al.*, 1993) it remains unclear whether the sole upregulation of HSF1 in tumors overexpressing HER2 is sufficient to activate HSF1-driven transcription. Tang and co-workers (2015) demonstrated that malignant cells use the RAS-RAF-MEK signaling pathway not only to promote their growth but also to preserve tumor proteostasis in an HSF1-dependent manner. They showed that phosphorylation of HSF1 on S326 by MEK enhances HSF1 *trans*-activation capacity, which protects tumor cells from the toxic amyloid formation. However, it is unknown whether other PTMs of HSF1 are needed for its activation in cancer.

### 3.3.5. HSF1 in proteinopathies

Protein aggregation is considered as a hallmark of neurodegeneration. Neurodegenerative diseases are pathologically associated with aggregates of specific proteins, such as α-synuclein in Parkinson's disease, huntingtin in Huntington's disease (HD), TDP-43 and SOD1 in amyotrophic lateral sclerosis (ALS), and amyloid precursor protein (APP) in Alzheimer's disease (reviewed in Ross and Poirier, 2004).

HSPs as molecular chaperones guide proteins from their production at the ribosomes to different stages to assist their folding and, if not possible, assist in their degradation (**Figure 11**). Furthermore, HSPs are able to prevent the initiation of protein aggregation, facilitate the removal of aggregates and ameliorate their toxic effects in various models of neurodegenerative disorders (reviewed in Kampinga and Bergink, 2016). Since the majority of HSPs are under the transcriptional control of HSF1, exogenous activation of HSF1 could serve as a promising strategy to combat neurodegeneration. Indeed overexpression or chemical activation of HSF1 has been shown to reduce protein aggregation and ameliorate cytotoxicity in multiple cellular and organismal models (Fujikake et al., 2008; Fujimoto et al., 2005; Katsuno et al., 2005; Kim et al., 2016; Labbadia et al., 2011; Neef et al., 2010; Verma et al., 2014).

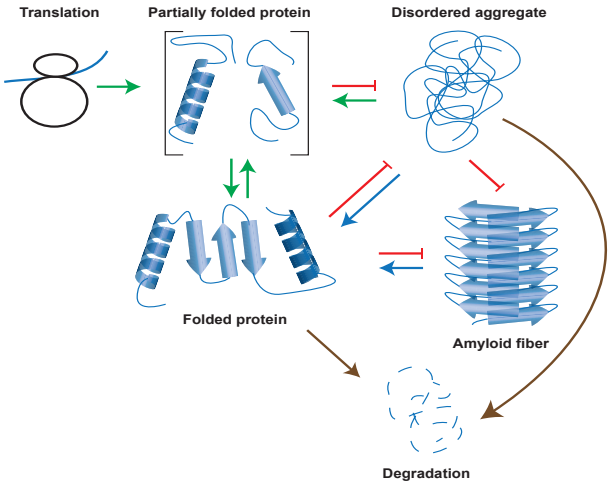


**Figure 11. From protein synthesis to degradation.** Heat shock proteins (HSPs) assist proteins from their synthesis in ribosomes to their correctly folded state (green arrows). Alternatively, if the correct folding is not possible, HSPs direct damaged proteins to degradation (brown arrows). HSPs prevent the formation of protein aggregates at different stages, either by blocking the formation of aggregates (in red) or through their disaggregation capacity (blue arrows).

Two recent studies have revealed that in neuronal cells exposed to aggregation-prone proteins, such as α-synuclein or huntingtin, HSF1 protein was degraded by the ubiquitin-proteasome system (Gomez-Pastor *et al.*, 2017; Kim *et al.*, 2016). α-synuclein aggregation resulted in increased ubiquitination of HSF1, mediated by a ubiquitin E3 ligase NEDD4 (neural precursor cell expressed developmentally down-regulated protein 4). Intriguingly, treatment of cells with resveratrol, or overexpressing SIRT1, in the presence of α-synuclein aggregates, prevented HSF1 degradation (Kim *et al.*, 2016). This indicates the existence of functional cross-talk between HSF1 PTMs that affects protein stability and subsequently HSF1 *trans*-activation capacity. In HD another pathway mediating the proteasomal destruction of HSF1 was reported

(Gomez-Pastor *et al.*, 2017). Cells expressing aggregation-prone huntingtin protein and a mouse model of HD, upregulate casein kinase 2α-prime (CK2α′) and E3 ubiquitin ligase FBXW7α. CK2α′ phosphorylates HSF1 on S303 and S307 which targets HSF1 for degradation in an FBXW7α-mediated manner resulting in decreased expression of HSPs and huntingtin aggregation in cells. *ck2α′*$^{+/-}$ HD mouse model displayed unaltered HSF1 protein levels and reduced huntingtin aggregation in the striatum. Furthermore, stabilization of HSF1 levels in CK2α′ heterozygote HD mice ameliorated the neurobiological defects of HD and restored the normal body mass of animals. Taken together the currently available knowledge of HSF1 in neurodegeneration, HSF1 can serve as a promising target for the development of drugs aimed at reducing pathogenic protein aggregation.

# AIMS OF THE STUDY

HSR is one of the most well-known cellular stress responses. Stress-inducible transcription of *HSP70* gene has served as a model for studying transcription for decades. Multiple laboratories focused on understanding how the HSR is regulated, by investigating HSF1, the master regulator of HSR. HSF1 is a unique transcription factor in many aspects, for example, it forms trimers rather than dimers, and its activity is mainly regulated by PTMs. HSF1 stress-inducible phosphorylation has been reported to occur in multiple species, such as yeast, mouse, and human. Phosphorylation as the most prominent PTM was thought to be a central regulatory mechanism controlling HSF1 activation. This hypothesis was supported by a finding that DNA-bound HSF1, which is not phosphorylated to the same extent as upon exposure to heat stress, is not capable of activating gene expression (Jurivich *et al.*, 1995). In addition, mutations of phosphorylation sites within the RD of the constitutively bound chimeric HSF1 protein resulted in de-repression of HSF1 activity under control conditions (Knauf *et al.*, 1996). Despite extensive studies on the HSF1 phosphorylation, no conclusive answer has been obtained, whether phosphorylation is required in the transition from an inactive to a transcriptionally competent HSF1. Thus, in the first study, we wanted to determine the impact of phosphorylation on the HSF1-mediated activation and control of the HSR.

Activation of the HSR is a promising therapeutic strategy for multiple proteinopathies. However, many HSF1 activators found in various chemical screens, yield unwanted side-effects which hamper their progress into the clinics. Chaperone co-inducers are chemicals, which do not activate the HSR alone but potentiate the HSR initiated by other stimuli, mainly due to their ability to prolong HSF1 activation (Hargitai *et al.*, 2003, Westerheide et al., 2009). The potent chaperone co-inducer BGP-15 has been shown to have beneficial effects in multiple human disease models and human patients suffering from diabetes. The molecular mechanisms by which BGP-15 enhances chaperone expression have not previously been established and were addressed in the second study.

The focus of my PhD thesis was to gain a deeper understanding of how the HSR is regulated, and the specific aims were:

1. To establish whether the stress-inducible phosphorylation of HSF1 is required for initiation of the HSR
2. To obtain insight into the mechanisms of action of the chaperone co-inducer BGP-15

## EXPERIMENTAL PROCEDURES

### 1. Plasmid constructs (I)

Plasmids encoding myc-his HSF1 WT, myc-his HSF1 K80R (in pcDNA3.1/myc-His(-)A), Gal4-VP16, β-galacatosidase, Gal4-driven luciferase and *HSPA1A*-driven luciferase have been described earlier (Holmberg *et al.*, 2001; Sadowski *et al.*, 1988; Westerheide *et al.*, 2009) The phosphorylation-deficient HSF1 mutant (HSF1Δ~PRD) was generated by replacing 15 phosphorylatable serine and threonine residues within the myc-His- HSF1 wild-type (WT) RD with alanines. Fourteen sites (S230, S292, S303, S307, S314, S319, S320, T323, S326, S338, S344, S363, S368, and T369) were mutated by sequential rounds of site-directed mutagenesis using a QuikChange site-directed mutagenesis kit (Agilent Technologies) according to the manufacturer's instructions. Mutation 15, T367A, was performed by DNA Express, Inc. Gal4-VP16-HSF1 WT and Gal4- VP16- HSF1Δ~PRD were generated by cloning the regulatory domain (amino acids [aa] 220 to 389) of myc-His-HSF1 WT or myc-His-HSF1Δ~PRD into EcoRI-linearized pSGVP plasmid (pSGVP was kindly provided by Richard I. Morimoto, Northwestern University, Evanston, IL) by using an In-Fusion HD cloning kit (Clontech). The constructs were confirmed by sequencing.

### 2. Cell culture and treatments (I-II)

All cell lines were maintained at 37°C in a humidified 5% $CO_2$ atmosphere. Human adenocarcinoma cancer HeLa cells and human erythroleukemia K562 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) supplemented with 10% fetal calf serum (FCS, Gibco), 2 mM L-glutamine (Sigma-Aldrich), and antibiotics streptomycin (100 μg/ml) and penicillin (100 U/ml) (both from VWR). Mouse embryonic fibroblasts (MEFs) and *hsf1*$^{-/-}$ MEFs (McMillan *et al.*, 1998) were cultured in DMEM containing 10% FCS, 10 mM MEM non-essential amino acids (Sigma-Aldrich), 2 mM L-glutamine, and antibiotics.

Heat shock was induced by submersion of cells into a water bath at 39, 40, 41, 42 and 43°C for the indicated times. To cause heavy metal stress, $CdSO_4$ (Sigma-Aldrich), dissolved in sterile water was used at a concentration of 40 and 60 μM for the indicated times. BGP-15 was purchased from N-gene and cells were pre-treated with 10 μM BGP-15 for 1 h. Trichostatin A (TSA, Sigma-Aldrich) was dissolved in DMSO and treatment was done with 1 μM for 1 h.

### 3. Transfections (I)

For transient expression experiments, approximately $6x10^6$ cells were resuspended in 400 μl OptiMEM (Gibco) together with plasmid DNA. Cells were electroporated with single electric pulse (220 V, 975 μF for HeLa cells; 280 V, 975 μF for MEFs) in 0.4 μM BTX cuvettes using Bio-Rad Gene Pulser and allowed to recover for 48 h prior to further treatments.

**4. Immunoblotting (I-II)**

Cells were lysed in radioimmunoprecipitation assay lysis buffer (1% NP-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 0.15 M NaCl, 0.01 M sodium phosphate [pH 7.2], and 2 mM EDTA [pH 8]) supplemented with 0.5 mM phenylmethylsulfonyl fluoride and 1X protease inhibitor cocktail (Roche and Thermo Science) for 10 min on ice. Cell lysates cleared by centrifugation (15,000 X *g* for 10 min at 4°C), were boiled in Laemmli sample buffer, resolved on a sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and transferred to nitrocellulose membrane (Pierce) using a semi-dry transfer apparatus (Bio-Rad).

Immunoblotting was performed using standard protocols with 3% milk as a blocking agent. The following antibodies were used for immunoblotting: anti-HSF1 (AB-4; Thermo Scientific and SPA-901; Enzo Life Sciences), anti-HSF1-S303-P (Ab47369, Abcam), anti-HSF2 (3E2; Millipore), anti-$\alpha$-Actin (AC-40; Sigma-Aldrich), anti-Hsc70 (SPA-815; Enzo Life Sciences), anti-VP-16 (V4388; Sigma-Aldrich). Secondary antibodies linked to horseradish peroxidase were from Promega and GE Healthcare and proteins were visualized using an enhanced chemiluminescence reagent (Thermo Scientific).

**5. Luciferase reporter assay (I)**

Transiently transfected cells were snap frozen and lysed in passive lysis buffer (Promega) for 10 min on ice. Cell lysates were cleared by centrifugation (15,000 x g for 10 min at 4°C) and the firefly luciferase activity produced by Gal4-driven luciferase plasmid was measured using a Luminoskan Ascent microplate luminometer (Thermo Scientific) with a luciferase assay reagent (Promega) as a substrate. The luciferase activity was normalized using Rous sarcoma virus promoter-driven β-galactosidase as an internal control by incubating the cell lysates in 100 mM phosphate buffer (pH 7.0) with 0.67 mg of ortho-nitrophenyl β-D-galactosidase (ONPG, Sigma-Aldrich)/ml, 1 mM $MgCl_2$ and 45 mM β-mercaptoethanol at 37°C for 1 h. The absorbance was measured at 420 nm.

**6. Protein dephosphorylation and protein turnover analyses (I)**

For protein dephosphorylation analysis, lambda protein phosphatase (λPP; New England BioLabs) was used according to the manufacturer's instructions. Briefly, transfected MEFs were subjected to a 30-min heat shock at 43°C and lysed in buffer C (25% glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, and 20 mM HEPES [pH 8]), and λPP was used at a concentration of 50 U/μg of whole-cell lysate. Samples were incubated at 30°C for 30 min, and the reaction was stopped by boiling in Laemmli sample buffer. To measure the protein turnover, transfected MEFs were treated for up to 15 h with cycloheximide (CHX; Sigma), which was added to the culture medium at a concentration of 20 μg/ml.

## 7. Chromatin immunoprecipitation (I, II)

A total of $5x10^7$ transfected MEFs were cross-linked immediately after treatment for 10 min with a final concentration of 1% formaldehyde, followed by quenching in 125 mM glycine. After lysis in Joost lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl [pH 8]), chromatin was sonicated using a Bioruptor (Diagenode), and 1 mg of whole-cell extracts was used for each immunoprecipitation. Samples were precleared using a 50% slurry protein G-Sepharose beads (GE Healthcare Life Sciences). Immunoprecipitation was performed overnight at 4°C using antibodies against HSF1 (SPA-901; Enzo Life Sciences). Normal rabbit serum (Jackson ImmunoResearch) was used as a non-specific antibody control. After washing of the immunocomplexes, the remaining proteins and RNA were digested by using proteinase K and RNase A. Cross-links were reversed by incubating the samples overnight at 65°C. DNA was purified with phenol-chloroform. Samples were analyzed by quantitative PCR using StepOnePlus or QuantStudio 12K Flex Real-Time PCR Systems (both from Applied Biosystems). The list of primers used for qPCR is shown in **Table 1.**

**Table 1.** Primers used in ChIP and MNase experiments

Forward primer (F), reverse primer (R).

| Gene | Amplicon name | Amplicon range relative to TSS of the gene | Primer sequences |
|---|---|---|---|
| HSPA1A | -1100 | From -1142 to -1070 | F: 5'-TAGTAGGCGGGCCCCAACACC-3'<br>R: 5'-GCATCTTGCCTCACAGTGCCC-3' |
| | -150 | From -241 to -57 | F: 5'-CCCCACACCCTCCCCCTCAG-3'<br>R: 5'-CTGGGCCAATCAGCGAGCCG-3' |
| | TSS | From -77 to +13 | F: 5'-CGGCTCGCTGATTGGCCCAG-3'<br>R: 5'-GTCAGCGTCTGGTGCCCTGC-3' |
| | +1100 | From +1048 to +1147 | F: 5'-CGCTGTCGTCCAGCACCCAG-3'<br>R: 5'-ACAGCTCTTCGAACCGCGCC-3' |
| HSPB1 | -110 | From -171 to -56 | F: 5'-TGGGAATCGCTCCAGCTACCG-3'<br>R: 5'-AAGCTTGCAAAGGGGGCGGG-3' |
| Notch 4 | -900 | From -967 to -842 | F: 5'-CCCCAGCATCCTTTGTGGAG-3'<br>R: 5'-GCCTCCAGACTCGTGGTAAA-3' |
| | TSS | From -66 to +48 | F: 5'-ACACACACACCAACCTCTCG-3'<br>R: 5'-GCAGGCTCAGGAGGAAGAAG-3' |
| | +500 | From +449 to +523 | F: 5'-GCCAGCTGGTTCTGAAGTAAG-3'<br>R: 5'-GACTGCCTGATATGGGGAAGAG-3' |
| Daxx | TSS | From -32 to +70 | F: 5'-CTTCCGGCTCTAAGCGGCCTG-3'<br>R: 5'-TCTGTTGTGGGGTCTGCGGT-3' |
| | +1100 | From +1058 to +1163 | F: 5'-TGCTCAACCAGGGCCCTCCAA-3'<br>R: 5'-TCCGAGCCCCGTGTGGATGG-3' |

## 8. Electromobility shift assay (I)

Cells lysed in buffer C were incubated with $^{32}$P-labeled oligonucleotides representing the proximal HSE of the human *HSPA1A* (*HSP70*) promoter. The protein-DNA

complexes were analyzed on a native 4% polyacrylamide gel, and the protein-DNA complexes were visualized by autoradiography.

**9. Immunofluorescence (I)**

Transfected HeLa cells were cultured on coverslips for 48 h before treatments. Treated and untreated cells were fixed in 3.7% paraformaldehyde and permeabilized with 0.5% Triton-X in phosphate-buffered saline (PBS) for 12 min, followed by blocking with 5% bovine serum albumin (BSA) in PBS for 1 h at room temperature. The cells were incubated with rabbit anti-HSF1 (Holmberg *et al.*, 2000) or mouse anti-myc (M4439; Sigma) antibodies overnight at 4°C, after which the unbound primary antibodies were washed off with PBS containing 0.1% Tween-20. After the washing step, the cells were incubated with secondary antibodies diluted 1:400 in 5% BSA-PBS for 1 h (donkey anti-rabbit antibody–Alexa Fluor 568 for anti-HSF1 and goat anti-mouse antibody–Alexa Fluor 488 for anti-myc, both from Life Technologies). Coverslips were mounted in Vectashield mounting medium with DAPI (4,6-diamidino-2-phenylindole; Vector Laboratories) for DNA staining. Immunofluorescence was performed with LSM 780 confocal microscope (Carl Zeiss, Inc.), and image analysis was performed using Fiji software (Schindelin *et al.*, 2012).

**10. Nuclear fractionation and HDAC activity assay (II)**

$8x10^6$ MEFs were used for HDAC activity assay. Cells were collected in cold PBS, and nuclear fractionation was conducted using the NE-PER Nuclear and Cytoplasmic Extraction Reagents kit (Thermo Fisher Scientific) according to the manufacturer's protocol. HDAC activity was measured using a commercially available, non-radioactive HDAC activity assay kit (Active Motif), according to the manufacturer's instructions. Briefly, 40 μg of nuclear lysates were incubated in HDAC assay buffer containing BOC-(Ac)Lys-pNi-troanilide for 60 min at 37°C. The reaction was stopped by adding the stop solution, and after adding the complete developing solution, the mixture was incubated for another 15 min at room temperature. Absorbance was measured at 405 nm using HIDEX Sense microplate reader.

**11. Quantitative real-time RT-PCR (I-II)**

Immediately after treatment, RNA was isolated with RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions and quantified using NanoDrop ND-1000 spectrophotometer (Thermo Scientific). 1 μg of total RNA was reverse transcribed with iSrcipt kit (Bio-Rad). Kapa Probe Fast Abi Prism qPCR kit (Kapa Biosystems) and SensiFast SYBR Hi-ROX (Bioline Reagents) were used for qRT-PCR reactions that were performed with StepOnePlus or QuantStudio 12k Flex Real-Time PCR System. The list of the primers and probes used for qRT-PCR is shown in **Table 2**. Relative quantities of the target gene mRNAs were normalized against their respective 18S RNA (RNA18S5). All reactions were run in triplicate from samples derived from at least four biological replicates.

**Table 2**. Primers and probes used in qRT-PCR experiments.

Forward primer (F), reverse primer (R), probe (P), dark quencher dye (BHQ). DNAJB1 amplification was done using SYBR green method.

| **Gene** | Primer and probe sequences |
|---|---|
| RNA18S5 | F: 5'-GCAATTATTCCCCATGAACG-3'<br>R: 5'-GGGACTTAATCAACGCAAGC-3'<br>P: 5'-FAM-TTCCCAGTAAGTGCGGGTC-BHQ-3' |
| HSPA1A/B | F: 5'-AGGTGCTGGACAAGTGCCAG-3'<br>R: 5'-AACTCCTCCTTGTCGGCCA-3'<br>P: 5'-FAM-CATCTCCTGGCTGGACTCCAACACG-BHQ-3' |
| HSPB1 | F: 5'-C ACTGGCAAGCACGAAGAAAG-3'<br>R: 5'-GCGTGTATTTCCGG GTGAAG-3'<br>P: 5'-FAM-ACCGAGAGATGTAGCCATGTT CGTCCTG-BHQ-3' |
| DNAJB1 | F: 5'-CTATGGAGAGGAAGGCCTGAA-3'<br>R: 5'-GGGTCTCCGTGGAATGTGTA-3' |

**12. *In vitro* HDAC activity assay (II)**

The inhibitory effect of BGP-15 on the HDAC1, 4, 6, 10, and SIRT1 was determined using full-length recombinant HDACs and a fluorogenic substrate (both from BPS Biosciences) according to the manufacturer's instructions. Briefly, the fluorogenic substrate was incubated with purified HDACs with various concentrations of BGP-15, TSA (for HDAC1, 4, 6, and 10) or nicotinamide (NAM) (for SIRT1) for 30 min at 37°C. The reaction was stopped by adding the developing solution, and the mixture was incubated for another 15 min at room temperature. Fluorescence intensity was measured with excitation and emission at a wavelength of 355/460 nm using HIDEX Sense microplate reader. Blank values were subtracted from the sample values and the samples without inhibitor were set to value 100. All reactions were run in quadruple using two different aliquots of inhibitors.

**13. Micrococcal nuclease (MNase) assay (II)**

MNase assay was modified from a previously described protocol (Elsing *et al.*, 2014). $1.5x10^7$ MEFs were crosslinked with 1% formaldehyde by incubating cells for 10 min at 37°C, after which 125 mM glycine was added for 5 min at 4°C. Cell pellets were washed and resuspended in TM2 buffer (10 mM Tris [pH 7.5], 2 mM $MgCl_2$, 1 mM DTT, 5 mM PMSF and 1x Pierce Protease Inhibitor). Samples were divided into two aliquots: one was digested with MNase (New England Biolabs) and the other was sonicated for 12 min using a Bioruptor (Diagenode). Samples were incubated with MNase at a final concentration of 6.3 U/µl for 10 min at 37°C, after which the reaction was stopped by adding 5% SDS and 50 mM EGTA. 0.2 M NaCl was added, and cross-links were reversed by incubating samples at 65°C overnight. Samples were treated

with RNase A (6 μg/ml) and proteinase K (50 μg/ml). DNA was purified with phenol-chloroform. Samples were analyzed by qPCR QuantStudio 12K Flex Real-Time PCR Systems. The list of primers used for qPCR is shown in **Table 1**. The enrichment of MNase-digested DNA was normalized to sonicated DNA. Values were compared with non-treated MNase resistance for the transcriptional start site (TSS) region of each of the analyzed loci which was set to value 1.

**14. Cell viability assay (II)**

MEFs were grown on 96-well white, clear bottom, tissue culture plates (Perkin Elmer) at density $5x10^4$ cells per well. Cells were either exposed to heat shock at 42°C or 45°C, and analyzed immediately after heat shock or left to recover for 16 h at 37°C or left untreated. Culture media were aspirated and 1X Calcein AM (Invitrogen) diluted in PBS was added to the cells and samples were incubated for 30 min at 37°C. Fluorescence intensity was measured with excitation and emission at a wavelength of 485/535 nm using HIDEX Sense microplate reader. Blank values were subtracted from the sample values and the viability of non-treated non-heat-shocked samples was set to value 100. All reactions were run in quadruple.

# RESULTS AND DISCUSSION

## 1. Phosphorylation as a regulatory mechanism of HSF1 transcriptional activity (I)

HSF1 activation is a multistep process, which involves trimerization, translocation to the nucleus, gain of DNA-binding and transcriptional activity. Early on it was observed that in mammalian cells, HSF1 undergoes massive phosphorylation events upon stress which correlate with a gain of its transcriptional capacity (Kline and Morimoto, 1997; Sarge *et al.*, 1993; Xia and Voellmy, 1997). Furthermore, HSF1 phosphorylation upon stress can be easily detected using immunoblotting as a slower migrating HSF1 on the SDS-PAGE and is widely used as a marker for HSF1 activation. It has been shown, that using sodium salicylate can cause HSF1 to trimerize and bind to DNA, but it is transcriptionally inactive (Jurivich *et al.*, 1995). Intriguingly, this salicylate-induced form of HSF1 was not phosphorylated like the heat-induced HSF1, indicating that transcriptional incompetency of salicylate-induced HSF1 may be due to lack of specific PTMs. In addition, mutations of specific phosphorylation sites within the RD cause the constitutively DNA-bound Gal4-HSF1 protein to become transcriptionally active in the absence of stress (Knauf *et al.*, 1996). This suggests that HSF1 phosphorylation plays a role in a transition from inactive to transcriptionally active HSF1 protein. Although multiple studies have focused on understanding the nature of stress-inducible phosphorylation on HSF1 activity (Guettouche *et al.*, 2005; Hietakangas *et al.*, 2003; Holmberg *et al.*, 2001; Xu *et al.*, 2012), no clear answer to the role of HSF1 phosphorylation during its activation has been obtained. In this project, we tested whether the stress-inducible phosphorylation within the RD of HSF1 is necessary to gain its *trans*-activation capacity.

### 1.1. Generation and characterization of a phosphorylation-deficient HSF1 mutant

To address the question regarding the role of HSF1 phosphorylation during stress, we generated a phosphorylation-deficient mutant of human HSF1 (HSF1Δ~PRD), where all known 15 phosphorylation sites within the RD were mutated to non-phosphorylatable alanines (**Figure 12**). We focused on the RD since this part of HSF1 harbors ~70% of the HSF1 phosphorylation sites (Xu *et al.*, 2012) and has been shown to be indispensable in the transition of inactive to transcriptionally active HSF1 (Green *et al.*, 1995; Newton *et al.*, 1996).

Mutation of multiple residues within a protein can change its biochemical properties, such as stability and localization (Gromiha, 2007; Laurila and Vihinen, 2009; Yutani *et al.*, 1987). Thus, before we performed the functional studies, we first addressed whether the removal of phosphorylation within the RD affects HSF1 expression, stability, and localization. To accomplish this, we expressed HSF1Δ~PRD, HSF1 Wt and an empty plasmid in *hsf1*$^{-/-}$ mouse embryonic fibroblasts (MEFs). In the majority of our experiments, we used *hsf1*$^{-/-}$ MEFs to prevent endogenous HSF1 interactions from influencing the experimental results.

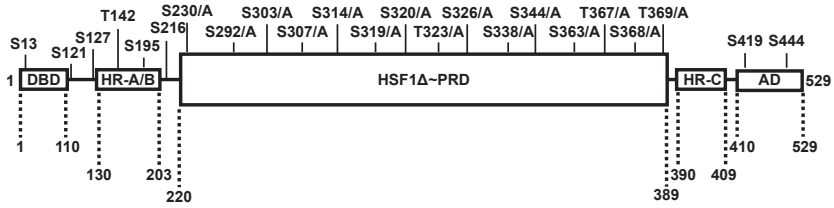


**Figure 12. A model of HSF1Δ~PRD, a phosphorylation-deficient HSF1 mutant.** In HSF1Δ~PRD, 15 phosphorylation sites in the regulatory domain (RD) were mutated from serine (S) and threonine (T) residues to alanines (A) as indicated. In addition, non-mutated, phosphorylation sites outside the RD are shown. Other HSF1 domains include the DNA-binding domain (DBD), heptad repeat domains (HR-A/B and HR-C), and activatory domain (AD). The figure is not drawn to scale.

We found that the generated mutant is expressed and that it migrates faster on the gel than HSF1 Wt in control conditions (I, Fig 1B, lane 5 *vs.* 7 and Fig 1C, lane 3 *vs.* 5). This indicates that the RD is heavily phosphorylated already in control conditions. Moreover, treatment of cell lysates with λ protein phosphatase did not result in further change in the migration pattern of HSF1Δ~PRD from unstressed cells (I, Fig 1C, lane 5 *vs* 11) which differed from the results obtained for HSF1 Wt when the same treatment was applied (I, Fig 1C, lane 3 *vs* 9). It implies that during control conditions HSF1 is mainly phosphorylated within the RD. However, the presence of individual phosphorylated sites outside the RD cannot be excluded. We also observed that upon heat shock HSF1Δ~PRD migrates more slowly on the gel than in control conditions (I, Fig 1B, lane 7 *vs.* 8 and Fig 1C, lane 5 *vs.* 6). Treatment with λ protein phosphatase caused HSF1Δ~PRD from unstressed and stressed cells to migrate similarly (I, Fig 1C, lane 11 *vs.* 12), indicating that HSF1Δ~PRD retained some ability to undergo stress-inducible phosphorylation. This is not unexpected, as there are known phosphorylation sites present outside the RD (**Figure 12**). Also, mutation of phosphorylation sites within the RD might lead to activation of unknown secondary phosphorylation sites within the RD. While we recognized that these other phosphorylation sites might influence experimental results, the fact that the RD has been shown to be critical for HSF1 activation and combined with our observation that HSF1Δ~PRD displayed a substantial reduction in phosphorylation under both stress and unstressed conditions were sufficient evidence for us to decide to proceed with further experiments.

We also examined HSF1 protein turnover using the cycloheximide treatment, which prevents protein synthesis (Obrig *et al.*, 1971), which is the most common approach to determine turnover or half-life of protein in cultured cells. The turnover of HSF1Δ~PRD was similar to that of HSF1 Wt (I, Fig 1D), showing that HSF1 phosphorylation within the RD does not regulate its stability. This is most likely the case for HSF1 phosphorylation in general, since Raychaudhuri and co-workers have reported that HSF1 stability is regulated mainly through acetylation (Raychaudhuri *et al.*, 2014). In their study, downregulation of acetyltransferase EP300 markedly reduced

HSF1 protein levels under control and stress conditions, suggesting that acetylation stabilizes HSF1 protein levels.

In normal conditions, HSF1 can be detected both in the cytoplasm and nucleus. In response to stress stimuli, HSF1 accumulates in the nucleus, where it drives transcription of its target genes. It has been previously demonstrated that phosphorylation of HSF1 influences cellular localizations, where the phosphorylation of S320 by protein kinase A (PKA) retains HSF1 in the nucleus (Murshid *et al.*, 2010). Furthermore, it was proposed that phosphorylation of S303 and S307 would allow for 14-3-3ε-mediated nuclear export of HSF1 (Wang *et al.*, 2003c). However, these results are controversial, since another study reported no difference in subcellular localization of wild-type HSF1 and S303A HSF1 muntant (Hietakangas *et al.*, 2003). In mammalian cells upon stress, HSF1 forms specific subnuclear structures, called nuclear stress bodies (nSBs) (Biamonti and Vourc'h, 2010). As shown previously, to form nSBs HSF1 must be able to localize to the nucleus and be DNA-binding competent (Alastalo *et al.*, 2003). We used nSBs to test HSF1Δ~PRD ability to localize into the nucleus and to bind DNA under stress conditions. To accomplish that, we transiently expressed HSF1Δ~PRD, HSF1 Wt and an empty plasmid in HeLa cells, exposed them to an acute heat shock and, using immunofluorescence, we examined the subcellular localization of HSF1 by confocal microscopy.

In unstressed HeLa cells, both HSF1 Wt and HSF1Δ~PRD were predominately localized in the nucleus (I, Fig 2). This is in agreement with previously published results from our laboratory, where HSF1 was observed predominantly in the nucleus already in control conditions (Alastalo *et al.*, 2003). When exposed to heat stress both HSF1 Wt and HSF1Δ~PRD altered their localization pattern and formed nSBs (I, Fig 2). Taken together, we demonstrated that HSF1 phosphorylation within the RD does not change the cellular localization of HSF1 under normal and stress conditions and is not required for formation of nSBs. These results introduce a discrepancy with previously published observations regarding the role of phosphorylation in the HSF1 localization where the mutation of S320 results in export of HSF1 from the nucleus while mutation of S303 or S307 prevented HSF1 nuclear export. One possibility is that the previous studies utilized an HSF1 protein with either single or double point mutations, while our construct has 15 sites mutated simultaneously. Hence, it is plausible that in HSF1Δ~PRD the effect of the single site mutation is being negated by the mutation of the other site. Reintroduction of S320 and S303 or 307 into HSF1Δ~PRD could allow for validation of the previously reported effect of phosphorylation on HSF1 localization.

### 1.2. DNA-binding capacity of HSF1 is not regulated by phosphorylation

Previous studies showed that specific chemical compounds, such as sodium salicylate, can induce binding of HSF1 to DNA, without gain of its transcriptional activity (Jurivich *et al.*, 1992). Salicylate-induced HSF1 is not phosphorylated in a manner similar to that observed for the HSR. However, there is still a change in

phosphorylation of HSF1 compared to control conditions (Jurivich *et al.*, 1995). Thus, it is plausible that phosphorylation contributes to HSF1 DNA-binding activity. Using immunofluorescence, we observed that HSF1Δ~PRD is able to form nSBs (I, Fig 2), which indicated DNA-binding competency. However, it is possible that HSF1Δ~PRD is localized to the nSBs *via* endogenous HSF1. To test whether HSF1Δ~PRD is directly capable of binding DNA in the context of chromatin we performed ChIP in *hsf1*$^{-/-}$ MEFs expressing either HSF1 Wt, HSF1Δ~PRD or an empty plasmid. Using ChIP, we found that the amount of HSF1Δ~PRD bound to the promoter regions of *HSPA1A* and *HSPB1* upon acute heat stress were the same as HSF1 Wt (I, Fig 3A). This indicates that phosphorylation does not regulate HSF1 binding to DNA in either unstressed cells or in the early phase of the HSR.

ChIP in transfected MEFs is technically challenging due to low protein concentration in cells, which requires a high number of cells for each experiment. Thus, in transfected MEFs EMSA, which measures the ability of protein to bind naked DNA containing specific regulatory sequences, allows for easier analysis of multiple time points and/or treatments. Using EMSA, we analyzed HSF1Δ~PRD DNA-binding competency during prolonged stress. For this purpose, we treated *hsf1*$^{-/-}$ MEFs expressing either HSF1 Wt, HSF1Δ~PRD or an empty plasmid with cadmium sulfate (60 μM CdSO4). Cadmium is a heavy metal, which has been shown to induce HSF1-dependent HSPs expression (Murata *et al.*, 1999), and based on our observations *hsf1*$^{-/-}$ MEFs tolerate prolonged exposure to cadmium better than to heat stress. Whole cell extracts were incubated with the $^{32}$P-labeled oligonucleotides containing the proximal HSE of the *HSPA1A* promoter. During prolonged exposure to cadmium (up to 9 hours), the HSF1Δ~PRD DNA-binding pattern was similar to that of HSF1 Wt (I, Fig 3B). HSF1Δ~PRD and HSF1 Wt binding to HSE-containing oligonucleotides were also observed during the recovery from stress (I, Fig 3B), which indicates that the attenuation pattern in HSF1Δ~PRD is not altered. Taken together, our results show that phosphorylation within the RD does not affect HSF1 binding under normal or stress conditions or in the recovery from stress.

In our study, we examined HSF1Δ~PRD binding capability to HSEs in general by EMSA and actual binding of HSF1Δ~PRD to *HSPA1A* promoter using ChIP. However, during heat shock HSF1 binds to over 1200 *loci* in K562 cells (Vihervaara *et al.*, 2013), and to over 1500 *loci* in testis (Korfanty *et al.*, 2014). Furthermore, in control conditions, HSF1 occupies 45 genomic *loci* in K562 cells (Vihervaara *et al.*, 2013), while almost 1500 *loci* in testis (Korfanty *et al.*, 2014). These observations indicate selectivity in HSF1 target sites in different tissues and conditions. Given recent advancements in the high-throughput sequencing technologies, it would be beneficial to test HSF1Δ~PRD DNA-binding on the genome-wide scale using ChIP-seq. This approach would reveal whether phosphorylation contributes to selecting the genes containing HSEs where HSF1 binds. Interestingly, in a recent study, the effect of phosphorylation on the DNA-binding of the *S. cerevisiae* HSF1 (ScHSF1) was analyzed by ChIP-seq (Zheng *et al.*, 2016). They generated an HSF1 phosphorylation-deficient mutant, where 152 serine

and threonine (S/T) residues were mutated to alanines (all S/T residues in the protein except one). Using that mutant, they showed that phosphorylation-deficient ScHSF1 displayed unaltered DNA-binding activity across the genome, yet, it is possible that HSF1 phosphorylation in mammals might affect HSF1-binding sites for other physiological functions of HSF1, such as development.

### 1.3. Phosphorylation regulates the magnitude of HSR

Having established that mutations of 15 phosphorylation sites do not change the biochemical properties of HSF1Δ~PRD, we proceeded to test whether stress-induced phosphorylation within the RD is required for HSF1 activation. We compared the mRNA levels of *HSP70* (*HSPA1A/B*) and *HSP25* (*HSPB1*) in MEFs expressing either HSF1 Wt or HSF1Δ~PRD. *HSP70* and *HSP25* levels were similar in MEFs expressing HSF1 Wt or HSF1Δ~PRD under control conditions, which indicates that HSF1Δ~PRD is not spontaneously activated (I, Fig 4A,B). Earlier studies suggested that removing certain phosphorylation sites from the RD would cause HSF1 activation without stress (Knauf *et al.*, 1996). However, in those studies, authors used an HSF1 mutant, where both the HSF1 DBD and oligomerization domain had been replaced with the Gal4 DBD. This mutant was constitutively bound to a promoter containing four repeats of the GAL upstream activation site (Keegan *et al.*, 1986). Since HSF1Δ~PRD cannot bind HSE without stress (I, Fig 3A,B), we concluded that the loss of phosphorylation within the RD of full-length HSF1 does not lead to the spontaneous activation of HSF1.

Next, we analyzed the ability of HSF1Δ~PRD to drive stress-inducible expression of HSPs. We exposed *hsf1*$^{-/-}$ MEFs expressing either HSF1 Wt or HSF1Δ~PRD to acute heat stress. To our surprise, HSF1Δ~PRD was capable of inducing expression of HSPs (I, Fig 4A). This shows that stress-inducible phosphorylation of HSF1 within the RD is not necessary for HSF1 to become active. Moreover, cells containing HSF1Δ~PRD displayed 2-fold higher levels of HSPs mRNA when compared with cells expressing HSF1 Wt. This suggests that bulk phosphorylation of HSF1 in the RD suppresses its *trans*-activation capacity.

Elevated temperature is one of many stress stimuli which activate the HSR. It might be possible that HSF1 phosphorylation is essential for HSF1 activation in other types of stress, but not heat stress. Therefore, we repeated the experiment described above by exposing cells to heavy metal cadmium instead of heat shock. We found that HSF1Δ~PRD was also capable of inducing expression of HSPs, with HSP levels in cells expressing HSF1Δ~PRD being 2-fold higher than in cells expressing HSF1 Wt (I, Fig 4B). Taken together, our results demonstrate that phosphorylation within the RD is not necessary for the HSF1-mediated activation of the HSR. Furthermore, it appears that the primary function of HSF1 stress-induced phosphorylation within the RD is to limit the magnitude of the HSR, given the higher HSP mRNA levels in cells expressing HSF1Δ~PRD.

While the exact mechanism of how phosphorylation contributes to the *trans*-activating capacity of HSF1 is unknown, previous studies have indicated that phosphorylation

both positively and negatively contributes to HSF1-mediated gene expression (reviewed in Anckar and Sistonen, 2011). It is then plausible that the majority of phosphorylation sites within the RD are repressive in nature. It cannot be excluded that phosphorylation sites outside the RD are crucial for HSF1 activation. A study by Zheng and co-workers demonstrated that the phosphorylation-deficient ScHSF1 remained fully stress-inducible (Zheng *et al.*, 2016), which suggests that phosphorylation-independent HSF1 activation is likely a common phenomenon. However, in the same study, it was reported that the phosphorylation-deficient ScHSF1 was not capable of driving gene expression upon stress to the same extent as ScHSF1 Wt. We can only speculate on the mechanisms behind distinct *trans*-activation capacities of HSF1 in *S. cerevisiae* and mammals. One possible explanation is the existence of other HSF family members in mammals, such as HSF2, which can form heterotrimers with HSF1 (Sandqvist *et al.*, 2009). For instance, HSF1-HSF2 heterotrimers formed in the developing brain exposed to alcohol exhibit lower *trans*-activation capacity compared to homotrimers alone (El Fatimy *et al.*, 2014). Thus, it is possible that the functional outcome of phosphorylation on HSF1 transcriptional activity partially stems from controlling HSF1-HSF2 interactions and further studies should address this hypothesis.

Interestingly, ScHSF1 mutant where all 116 S/T residues that fall outside of the DNA-binding and trimerization domains were replaced with aspartate to mimic constitutive phosphorylation displayed enhanced *trans*-activation capacity under basal conditions, but not upon stress (Zheng *et al.*, 2016). One might speculate that mimicking phosphorylation on the mammalian HSF1 would result in its spontaneous activation. This is plausible, but unlikely, given the fundamental differences between ScHSF1 and mammalian HSF1. ScHSF1 is transcriptionally active under normal conditions, where it is required to drive transcription of HSPs and either loss or amorphic mutations of ScHSF1 results in cell death (Solís *et al.*, 2016). In contrast, mammalian HSF1 is not an essential protein under normal conditions (McMillan *et al.*, 1998), and basal expression of HSPs occurs in an HSF1-independent manner (Mahat *et al.*, 2016). Furthermore, mammalian HSF1 is not capable of replacing ScHSF1, due to its inability to trimerize and subsequent lack of DNA-binding capacity at the control conditions (Liu *et al.*, 1997). Only when mutations that force mammalian HSF1 into the constitutively trimeric state are introduced, mammalian HSF1 is able to replace ScHSF1 (Neef *et al.*, 2013). The mammalian HSF1 is under more strict control and requires more activation steps to become fully transcriptionally active. Thus, mimicking phosphorylation in mammalian HSF1 would not result in its activation, unless the circumstances for trimerization and DNA-binding activity would be bypassed. This hypothesis could be experimentally verified by exposing the HSF1 mutant that mimics constitutive phosphorylation to a sodium salicylate which induces binding of HSF1 to DNA, but not transcription of HSP genes. That should result in the inducible expression of HSPs by the HSF1 mutant mimicking constitutive phosphorylation in the absence of stress.

### 1.4. HSF1 activation threshold is defined by phosphorylation

After we established that the HSF1 RD phosphorylation is not required for HSF1 activation, a question remains how the lack of HSF1 phosphorylation can result in increased HSPs expression. It can either lower the threshold needed for HSF1 activation or increase the rate of transcription by Pol II. We tested the effect of HSF1 phosphorylation on the activation threshold. To do this, we exposed *hsf1*$^{-/-}$ MEFs expressing HSF1 Wt or HSF1Δ~PRD to the series of heat stress with temperatures ranging from 39°C to 41°C. It has been shown previously that moderate heat stress of 40°C can also cause HSF1 activation, but less efficiently than at 43°C (Shinkawa *et al.*, 2011; Wang *et al.*, 2003a). Exposure of up to a 60-minute heat shock at 39°C did not induce *HSP70* in cells expressing either HSF1 Wt or HSF1Δ~PRD (I, Fig 5A). Using 40°C heat stress, we observed a significant increase in *HSP70* mRNA levels at a 60-minute heat shock only in cells expressing HSF1Δ~PRD, whereas at 41°C both HSF1 Wt and HSF1Δ~PRD were capable of inducing *HSP70* mRNA (I, Fig 5A). However, in a 30-minute heat shock at 41°C, we observed increased levels of *HSP70* mRNA only in cells expressing HSF1Δ~PRD. We further verified our hypothesis by exposing cells to different concentrations of cadmium. Both HSF1 Wt and HSF1Δ~PRD were capable of inducing *HSP70* expression when cells were exposed to 60 μM cadmium (I, Fig 5B). However, when exposed to 40 μM cadmium, only HSF1Δ~PRD was capable of increasing *HSP70* expression. We conclude that HSF1 phosphorylation status determines the activation threshold for HSF1 and that phosphorylation prevents HSF1 activation in the presence of mild stress stimuli. Intriguingly, loss of HSF2 results in higher HSF1 binding and elevated HSP70 expression in MEFs exposed to mild stress at 40°C (Shinkawa *et al.*, 2011), which corresponds to our finding that HSF1Δ~PRD has a lower threshold for activation. Thus, the formation of HSF1-HSF2 heterotrimers could serve as a buffer for controlling the severity of the stress needed for outright activation of the HSR.

The stress-mediated transcription of HSPs is an extremely rapid and efficient process. It is established that in *Drosophila*, Pol II on the HSP70 gene is released into productive elongation every four seconds upon acute heat stress (Nechaev and Adelman, 2011). The exact kinetics of Pol II release in mammals is not known, but most likely it is not very different. It is possible that HSF1 phosphorylation affects the release speed of the Pol II into productive elongation. One way to measure the amount of transcriptionally engaged Pol II at the HSP promoters would be to perform native elongating transcript sequencing (NET-seq), which reveals the density of RNA polymerase across the genome (Churchman and Weissman, 2012). However, if the difference in Pol II amounts on the gene body is subtle, technical imperfections of ChIP, such as cross-linking efficiency or antibody performance, might not be able to distinguish between these changes. Another way to determine Pol II release would be by directly measuring transcription rates, with either nuclear run-on for single genes or in a genome-wide approach using, for example, precision nuclear run-on and sequencing

assays (Kwak *et al.*, 2013). This would allow for more precise quantitative and kinetic analyses of native transcript synthesis during the HSR.

### 1.5. Phosphorylation-deficient HSF1 regulatory domain is capable of sensing stress

Although the molecular basis of HSF1 activation has been studied in great detail, the mechanism by which cells mount the HSR in the first place is not well understood. It has been shown that HSF1 alone can form trimers and acquires DNA-binding capacity when exposed to heat, which demonstrates an intrinsic ability of HSF1 to sense stress (Goodson and Sarge, 1995; Hentze *et al.*, 2016; Larson *et al.*, 1995). A specific region of the HSF1 protein, subsequently reffered to as RD, was identified as responsible for keeping HSF1 inactive under normal conditions (Green *et al.*, 1995; Newton *et al.*, 1996). Phosphorylation has been proposed as one of the mechanisms allowing the RD to sense stress. This was evidenced by mutation of specific phosphorylation sites which greatly increased activity of the chimeric Gal4-HSF1 proteins under normal conditions (Knauf *et al.*, 1996). Intriguingly, the HSF1 RD is capable of providing heat regulation for heterologous activation domains (AD), such as the VP16 AD (Newton *et al.*, 1996). We, therefore, wanted to investigate whether the phosphorylation-deficient RD is able to provide heat inducibility for the VP16 AD in a similar manner as has been previously described. For this purpose, we generated chimeric constructs consisting of Gal4 DBD, RD (aa 220 to 389) from HSF1 Wt, HSF1Δ~PRD, and VP16 AD (I, Fig 6A). As a positive control, we used Gal4-VP16 which is a potent transactivator of genes containing Gal4-binding sites in their promoter regions (Sadowski *et al.*, 1988). We co-transfected the described constructs together with the Gal4-driven luciferase reporter gene into HeLa cells and measured the reporter activity.

In agreement with previous results, insertion of the wild-type RD into Gal4-VP16 resulted in decreased transcription of the reporter gene under normal conditions (I, Fig 6B). The presence of the phosphorylation-deficient RD had no effect on the reporter gene expression, since the luciferase activity was equal to that in cells expressing Gal4-VP16. This indicated that indeed, the previously identified phosphorylation of S303 and S307 is necessary for the repressive function of the RD under normal conditions (Knauf *et al.*, 1996). However, this applies only to the constitutively DNA-bound protein, since we did not observe the similar effect for the full-length HSF1 (I, Fig 4A,B). Next, we exposed cells to a 60-minute heat stress at 42°C followed by a 5-hour recovery. Repression of transcriptional capacity by the Wt RD was lifted upon heat stress, since the luciferase activity in cells expressing Gal4-VP16-HSF1 Wt corresponded to that observed in cells expressing Gal4-VP16 (I, Fig 6B). Intriguingly, luciferase activity in cells expressing Gal4-VP16 with the phosphorylation-deficient RD was even higher than in cells expressing Gal4-VP16. This result demonstrates that HSF1 RD deprived of phosphorylation is still capable of providing heat inducibility for the heterologous AD.

The phosphorylation-dependent sumoylation of the HSF1 RD is one possible mechanism that would regulate the stress-sensing capacity of HSF1. HSF1 undergoes

sumoylation at K298 already in the first minutes of proteotoxic insult. In contrast to phosphorylation, sumoylation of HSF1 is a transient modification and the amount of sumoylated HSF1 decreases during stress (Hietakangas *et al.*, 2003). Intriguingly, the severity of stress is positively correlated with the HSF1 desumoylation rate (Hietakangas *et al.*, 2006). In the same study, it was shown that mutations in the HR-C domain, that force HSF1 to form trimers in the absence of stress, are sufficient to cause HSF1 sumoylation already under basal conditions. Thus, it is highly plausible that sumoylation of the RD in Gal4-VP16-HSF1 Wt under control conditions leads to an observed decrease in transcription of the reporter gene (I, Fig 6B). Concurrently, mutations of phosphorylation sites, including S303, in the Gal4-VP16- HSF1Δ~PRD prevent sumoylation and result in loss of RD-mediated repression on the VP16 AD without stress. The question remains, how phosphorylation-deficient RD retains activity to increase *trans*-activation capacity of VP16 AD in a heat-inducible manner. While the phosphorylation of S303 has been shown to be necessary for HSF1 sumoylation, we cannot exclude that HSF1 can be sumoylated on K298 *via* different mechanisms. This notion is supported by previous findings using the Gal4-HSF1 chimeric protein, where alanine mutations of K298 and E300, which are key residues in HSF1 sumoylation motif, completely abolished heat inducibility (Knauf *et al.*, 1996). In turn, simultaneous mutation of S303 and S307 to alanines markedly increased transcriptional activity of Gal4-HSF1 chimeric protein without stress but did not result in complete loss of heat inducibility. However, another study showed that mutation of either K298 or S303 of HSF1 results in similar a de-repression of Gal4-HSF1 in the absence of stress (Hietakangas *et al.*, 2006). Thus, mutation of K298 in the Gal4-VP16-HSF1Δ~PRD chimeric protein would elucidate whether the residual sumoylation of the RD is responsible for the observed heat inducibility of the heterologous AD.

While there is evidence to support the model for sumoylation of K298 as a stress sensing mechanism for HSF1, many details of this mechanism remain unclear. For instance, most of the experiments on HSF1 sumoylation rely on overexpressing both SUMO and HSF1 proteins, which indicates that only small fraction of endogenous HSF1 undergoes sumoylation. A previous study that revealed sumoylation as a repressive modification of HSF1 *trans*-activation capacity used only proteasome inhibition as a stress, and a limited number of HSPs genes were analyzed (Hietakangas *et al.*, 2006). It would be of interest to investigate how the loss of HSF1 sumoylation affects the expression of genes regulated by HSF1 under various stress conditions and in the genome-wide scale using, for example, RNA sequencing technique. Thus, additional studies are needed to establish whether sumoylation could serve as a stress sensing mechanism.

### 1.6. HSF1 phosphorylation from a clinical perspective in human malignancies

A decade ago, fundamental discoveries about the importance of HSF1 in cancer were reported (Dai *et al.*, 2007; Min *et al.*, 2007). Dai and co-workers used a clinically relevant p53 mutation mouse model to show that mice lacking HSF1 have less solid tumors and increased survival when exposed to chemical skin carcinogenesis (Dai *et al.*, 2007).

Similarly, Min and co-workers reported that in $p53^{-/-}$ mice, which typically develop lymphomas, the loss of HSF1 resulted in a marked reduction of expected lymphomas (Min *et al.*, 2007). Further studies showed that cancer cells rely on HSF1 to maintain their malignant phenotype. Downregulation or inhibition of HSF1 has little effect on normal cells, but substantially reduce growth and survival of multiple cancer cell lines (Bustany *et al.*, 2015; Dai *et al.*, 2007; Dudeja *et al.*, 2011; Heimberger *et al.*, 2013; Shah *et al.*, 2015; Shah *et al.*, 2016). The combined results of these studies led to the emergence of HSF1 as an attractive therapeutic target to combat malignancy.

It is unknown how HSF1 turns from the guardian of proteostasis in non-transformed cells to the promoter of malignancy. HSF1 phosphorylation, specifically at S326, has been used as a marker for HSF1 activation in human cancers (Mendillo *et al.*, 2012; Shah *et al.*, 2016). The observation that HSF1 can be phosphorylated on S326 directly by MEK (Tang *et al.*, 2015) provides a link between aberrant alterations in the RAS/MEK signaling cascade and HSF1 activation in human malignancies, but only 30% of human cancers carry activating mutations in components of the RAS/MEK signaling cascade (Fernandez-Medarde and Santos, 2011). The results presented in this study, specifically show that HSF1 can be activated without phosphorylation within the RD, including S326, suggest that other mechanisms than phosphorylation might drive HSF1 activation in cancer. Furthermore, we have to acknowledge the limitations of our study. We used only a single non-transformed cell line, and we cannot exclude the possibility that the observed results are cell line specific. Nonetheless, our major finding that phosphorylation is not required for HSF1 activation was independently confirmed in *S. cerevisiae* (Zheng *et al.*, 2016). In addition, we used ectopically expressed HSF1 in cells devoid of endogenous HSF1. This setting prevents observations of long-term effects of HSF1Δ~PRD on the cell activity. During the course of this study, the CRISPR/Cas9 system emerged as a powerful genome editing tool (Gasiunas *et al.*, 2012). Using CRISPR/Cas9 it is possible to introduce single point mutations or genomic rearrangements into a particular target gene. Taking advantage of the CRISPR/Cas9 system allows for the generation of endogenously expressed phosphorylation-deficient HSF1 protein in cell, tissue, and organism. This approach would enable evaluation of the function of HSF1 phosphorylation not only in the HSR but also in other physiological and pathological conditions, where HSF1 plays an important role.

## 2. Molecular mechanisms of BGP-15-mediated chaperone co-induction (II)

Increasing HSP levels by activating the HSR is a promising therapeutic strategy in protein-folding disorders. Many chemical compounds which are able to activate the HSR yield unwanted side effects, *e.g.* result in protein damage due to their electrophilic nature. Chaperone co-inducers potentiate a pre-existing HSR and enhance the expression of inducible molecular chaperones when combined with cellular stress events, such as heat shock. Two well-known chaperone co-inducers, resveratrol and bimoclomol have been shown to augment the HSR by prolonging HSF1 binding to

DNA (Vígh *et al.*, 1997; Westerheide *et al.*, 2009). BGP-15 is another example of a chaperone co-inducer, however, its precise mode of action remains unknown. BGP-15 is a small molecule (350 Da) belonging to the group of hydroximic acids. It has been shown that BGP-15 intercalates into the plasma membrane where it stabilizes lipid rafts (Gombos *et al.*, 2011). In cells, BGP-15 treatment decreases amounts of ROS and inhibits PARP1 (Racz *et al.*, 2002; Sumegi *et al.*, 2017). BGP-15 administration has been shown to ameliorate disease-related pathologies in animal models of Duchene's muscular dystrophy, type II diabetes and ventilation-induced diaphragm dysfunction (VIDD) (Gehrig *et al.*, 2012; Henstridge *et al.*, 2014; Salah *et al.*, 2016). In those pathologies, beneficial effects of BGP-15 have been associated with increased levels of HSPs. Moreover, BGP-15 has an excellent safety profile in human patients, as shown in a clinical trial for type II diabetes (Literáti-Nagy *et al.*, 2009; Literáti-Nagy *et al.*, 2010). In this study, we aimed to elucidate the molecular mechanisms by which BGP-15 functions as a chaperone co-inducer.

### 2.1. BGP-15 accelerates HSF1 activation

The function of bimoclomol and resveratrol as chaperone co-inducers has been shown to rely on their ability to prolong HSF1 binding to DNA upon stress (Vígh *et al.*, 1997; Westerheide *et al.*, 2009). Resveratrol, due to its ability to activate SIRT1, maintains HSF1 in a DNA-binding competent state, by deacetylating the HSF1 DBD (Westerheide *et al.*, 2009). Hence, we first addressed the question whether the BGP-15 acts like resveratrol and bimoclomol during the HSR. For this purpose, we pretreated MEFs for 1 hour with 10 μM BGP-15, exposed them to 42°C heat shock up to 180 minutes and measured HSPs expression by qRT-PCR and HSF1 binding to the *HSP70* promoter by ChIP. BGP-15 alone is not capable of inducing the HSR, which can be observed as unaltered levels of *HSP70* and *DNAJB1* mRNA under control conditions (II, Fig 1A). Upon stress BGP-15 co-induces HSPs expression when compared with non-treated cells, but only up to 90 minutes of heat stress after which BGP-15 was not able to further induce HSPs mRNA levels (II, Fig 1A). HSF1 binding analysis revealed that in cells exposed to 120 and 180 minutes of heat stress, similar amounts of HSF1 were bound to the *HSP70* promoter in both BGP-15-treated and non-treated cells (II, Fig 1B). However, with BGP-15 treatment we noticed a marked increase in HSF1 binding at 60 minutes of stress when compared with the non-treated cells. These results suggest that BGP-15 does not co-induce the HSR by prolonging HSF1 activity, but it acts in the early phase of the HSR.

We decided to further investigate HSF1-mediated HSPs expression and HSF1 binding in the early phase of the HSR. To accomplish this, we treated MEFs as above, but this time we exposed them to a shorter, *i.e.* 15- and 30-minute, heat stress. As expected, BGP-15 increased *HSP70* and *DNAJB1* expression at 30 minutes when compared with non-treated heat-shocked cells (II, Fig 1C). Importantly, the *DNAJB1* mRNA levels were already higher after 15 minutes of heat stress in BGP-15-treated cells than in non-treated cells. DNA-binding analysis of HSF1 showed that more HSF1 occupies the *HSP70* promoter upon stress in cells pretreated with BGP-15 (II, Fig 1D). We concluded

that BGP-15 acts as a chaperone co-inducer *via* accelerating HSF1 activation in the event of proteotoxic stress.

The observation that HSF1 binding to the *HSP70* promoter at 90 minutes of heat stress in cells treated with BGP-15 is lower than in non-treated cells might indicate that BGP-15 accelerates attenuation of HSF1. Another HSR co-inducer, TPA, which acts as a PKC activator, is known to cause accelerated activation and attenuation of HSF1 (Holmberg *et al.*, 1997). However, the kinetics of HSF1-binding upon TPA exposure is different than reported above. In TPA-treated heat-shocked cells, HSF1 attenuation was already observed at 60 minutes of exposure to stress and could not be detected after 180 minutes of heat stress (Holmberg *et al.*, 1997). In the case of BGP-15 treatment, increased binding is maintained at 60 minutes of heat stress and HSF1-binding to DNA can also be observed at an 180-minute exposure to heat stress. The difference in the HSF1 binding kinetics might be the result of used methodology. The DNA-binding ability of HSF1 in TPA-treated cells was analyzed by Holmberg and co-workers (1997) using EMSA, which tests the ability of the whole cellular pool of HSF1 to bind "naked" DNA, while in our study we used ChIP which measures occupancy of HSF1 in the context of chromatin at a specific location on the genomic DNA. Nevertheless, the finding that HSF1 binding to the *HSP70* promoter is similar in both pretreated and non-treated cells at 120 and 180 minutes of heat shock (I, Fig 1B), suggests that global HSF1 attenuation is not accelerated. What we observed in BGP-15-treated cells can be defined as an accelerated entry into the attenuation phase, but not as a complete attenuation. Another result that supports this observation is that the mRNA levels of HSPs after 180 minutes of stress are similar in both BGP-15-pretreated and non-treated cells. Taken together, we have shown that BGP-15 accelerates HSF1 activation and increases HSPs expression in the early phase of HSR.

Induction of HSPs expression has been shown to exhibit a beneficial effect in various proteinopathies (reviewed in Kakkar *et al.*, 2014). It has been speculated that the protein damage, which occurs in certain pathologies, might not be severe enough to reach the threshold needed for the HSR activation (Voellmy, 2004). Hence, we examined whether BGP-15 can activate the HSR in the context of mild stress. For this purpose, we exposed MEFs to a febrile temperature of 40°C for 15, 30 and 60 minutes and measured *HSP70* mRNA levels. This temperature is capable of inducing the HSR, albeit kinetics and magnitude of the response are lower than at 42°C (II, Fig 1E *vs.* 1C,D). For instance, 5-fold induction of *HSP70* mRNA was observed after 30 minutes of heat stress at 40°C, whereas the exposure to 42°C led to a 20-fold induction after only 15 minutes of stress. Pretreatment of cells with BGP-15 accelerated HSF1-mediated HSPs expression also at the febrile temperature, and elevated levels of *HSP70* mRNA were detectable after only 15 minutes exposure to heat stress (II, Fig 1E). Furthermore, no increase in *HSP70* mRNA was detected at a 15-minute heat stress in non-treated cells. These results indicate that BGP-15 is capable of lowering the threshold for HSF1 activation and suggests a possible explanation for the increase in

HSPs levels observed upon BGP-15 treatment, as reported in previous studies of animal disease models (Gehrig *et al.*, 2012; Henstridge *et al.*, 2014; Salah *et al.*, 2016).

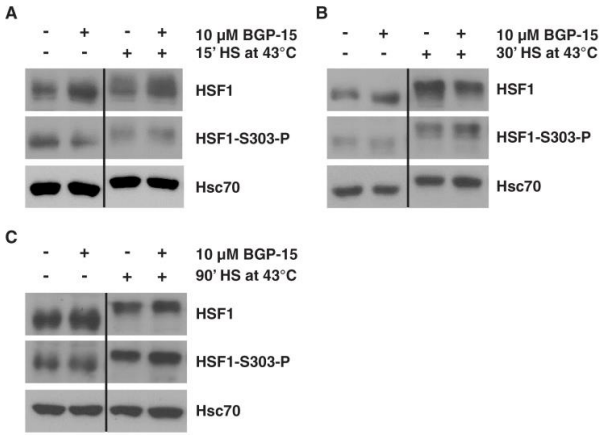


**Figure 13. BGP-15 does not alter the phosphorylation status of HSF1.** K562 cells were either pretreated with 10 μM BGP-15 for 1 h or not and exposed to a 43°C heat shock (HS) for 15 min (A), 30 min (B) or 90 min (C). Samples were analyzed by using Western blotting. Hsc70 is shown as a loading control. All immunoblots shown for each panel contain the samples from the same membrane and were cropped to show only relevant data.

In Study I, we showed that the HSF1 activation threshold is determined by the phosphorylation status of the HSF1 RD. Thus, it is possible that BGP-15 lowers the activation threshold of HSF1 *via* changes in the HSF1 phosphorylation. However, pretreatment of cells with BGP-15 did not modify the migration pattern of HSF1 on the SDS-PAGE under both control and stress conditions when compared to the non-treated cells (**Figure 13**), which indicates that overall HSF1 phosphorylation is not altered. Yet, it is possible, but unlikely, that presence of BGP-15 in cells changes phosphorylation of specific amino acid residues in HSF1 which determines the activation threshold. Hence, a comparative phosphoproteomic analysis of HSF1 from BGP-15-treated and non-treated cells could identify any differentially phosphorylated sites.

### 2.2. BGP-15 acts as an HDAC inhibitor

The DNA-binding activity of HSF1 has been shown to be regulated in an HDAC-dependent manner (Westerheide *et al.*, 2009; Zelin and Freeman, 2015). Resveratrol acts as a chaperone co-inducer because of its ability to stimulate the activity of SIRT1, which belongs to class III HDAC (Howitz *et al.*, 2003). However, a previous study showed that treatment with BGP-15 has no effect on sirtuins activity (Gombos *et al.*, 2011). Thus, we decided to test whether BGP-15 can modulate the activity of class I and II HDACs. We pretreated MEFs for 1 hour with 10 μM BGP-15 and compared HDAC activity using a commercially available assay. As a positive control, we used cells pretreated for 4 hours with 1 μM TSA, a well-known class I and II HDAC inhibitor (Yoshidas *et al.*, 1990). As expected, HDAC activity decreased in cells treated with TSA (II, Fig 2A). Surprisingly, treatment with BGP-15 also reduced HDAC activity, but the decrease was smaller when compared to the TSA treatment (15% *vs.* 30%). To date, although BGP-15 has been shown to interact with the lipid rafts in the plasma membrane (Gombos *et al.*, 2011), there is no evidence that BGP-15 can enter the cell. To

test whether the BGP-15-mediated reduction of HDAC activity requires interaction with the plasma membrane, we performed an HDAC activity assay on isolated nuclei. Nuclear extracts from MEFs were treated with 10 μM BGP-15 or 1 μM TSA for 1 hour at 37°C, and HDAC activity was assessed. In this assay both TSA and BGP-15 reduced HDAC activity by 35%, which indicates that the BGP-15 is capable of inhibiting nuclear HDACs.

Next, we examined whether BGP-15 is a direct HDAC inhibitor. We assessed HDAC activity using a fluorogenic acetylated substrate and purified HDACs belonging to class I (HDAC1), II (HDAC4, 6, and 10), and III (SIRT1). Class IV HDAC was not tested, since the only member of this class, HDAC11, is not expressed in MEFs (Gao *et al.*, 2002). As a positive control for HDAC inhibition, we used TSA for class I and II HDACs and nicotinamide (NAM) for class III HDACs (Bitterman *et al.*, 2002). We found that BGP-15 is not capable of inhibiting HDAC *in vitro*, in contrast to TSA and NAM (II, Fig 2C,D). These results indicate that BGP-15 is an indirect HDAC inhibitor capable of inhibiting HDACs activity in cells.

The mechanism by which BGP-15 reduces HDAC activity remains to be understood. HDAC activity can be regulated by controlling their expression levels in the cell. The members of a prominent Sp family of transcription factors have been shown to be the central regulators of HDACs expression (Liu *et al.*, 2006; Schuettengruber *et al.*, 2003; Zhang *et al.*, 2010). For instance, Sp1 and Sp3 occupy the distal promoter of the *HDAC1* gene in mice and are required for its expression (Schuettengruber *et al.*, 2003). Intriguingly, HDAC1 activity provides a feedback loop for controlling its expression; HDAC1 binds to its gene in the region containing Sp1 binding sites and inhibition of HDACs activity, including HDAC1 by TSA treatment or overexpression of HAT p300, results in increased expression of HDAC1 (Schuettengruber *et al.*, 2003). Furthermore, binding of Sp1 and Sp3 is observed on the HDAC4 and HDAC7 promoters, and a decrease in Sp protein levels inhibits HDAC4 and HDAC7 expression (Liu *et al.*, 2006; Zhang *et al.*, 2010). It remains to be elucidated if BGP-15 regulates HDAC protein levels. Given the short time of BGP-15 pretreatment (1 hour), it is unlikely that the HDAC levels would be altered. This assumption could be tested by verification of HDAC protein levels using immunoblotting in cells treated with BGP-15.

Class IIa HDACs consists of four members (HDAC4, 5, 7, and 9) that have been shown to be mainly regulated by its localization (reviewed in Di Giorgio and Brancolini, 2016). Class IIa HDACs are primarily active in the nucleus, thus their accumulation in the cytoplasm serves as a negative regulator of these proteins. Phosphorylation regulates nuclear localization of class IIa HDACs in a 14-3-3-dependent manner. For example, phosphorylation of HDAC4 and HDAC5 facilitates 14-3-3 interaction which masks the nuclear localization signal (NLS) and forces the cytoplasmic localization of these HDACs (Grozinger and Schreiber, 2000; Nishino *et al.*, 2008). Redistribution of class IIa HDACs is a quick response that allows for a prompt regulation of HDACs activity. It may be possible that BGP-15 affects the HDACs cellular localization. While direct evidence is still missing, the observation that pretreatment with BGP-15 decreases

HDACs activity in whole cell lysates (II, Fig 2A), suggests that BGP-15 inhibits the enzymatic activity of HDACs.

Specific multiprotein complexes, such as CoREST or nuclear receptor co-repressor (NCoR) complex, incorporate HDACs, as the central enzymatic component (Bantscheff *et al.*, 2011). In addition, HDAC activity in multiprotein complexes is often dependent on the activity of other HDACs. For example, HDAC4 is essential for deacetylation activity and transcriptional repression of target genes by the NCoR–SMRT–HDAC3 complex (Fischle *et al.*, 2002). Thus, it is highly plausible that BGP-15 targets non-HDAC subunits of the HDAC multiprotein complex, which interferes with their formation and activity. Future studies should address if BGP-15 interacts with any components of the HDAC multiprotein complexes. Adding a tag to BGP-15, *e.g.* biotin, would allow for immunoprecipitation of BGP-15 and identification of its cellular interaction partners *via* mass spectrometry.

**2.3. BGP-15 increases chromatin accessibility**

Inhibition of HDACs activity leads to a genome-wide alteration in gene expression (Chambers *et al.*, 2003; Glaser *et al.*, 2003; Peart *et al.*, 2005; Rafehi *et al.*, 2014). Use of HDAC inhibitors causes hyperacetylation of histones that neutralizes the lysine's positive charge on histones, which has the potential to weaken the interactions between histones and DNA (Bannister and Kouzarides, 2011). To test whether the BGP-15-mediated HDAC inhibition has an effect on the chromatin structure, we performed the micrococcal nuclease (MNase) DNA accessibility assay. The MNase is the most commonly used nuclease to assess nucleosome occupancy and chromatin accessibility and relies on the observation that DNA wrapped around the nucleosomes is resistant to MNase digestion (Nobile *et al.*, 1986). Treatment with BGP-15 decreased the MNase resistance throughout the *HSP70* promoter and coding region (II, Fig 3A). However, we cannot exclude the possibility that the observed acceleration of HSF1 activation is a combinatory effect of BGP-15-mediated HDAC inhibition on both HSF1 and chromatin. Performing EMSA and evaluating HSF1 binding to the "naked" DNA would provide evidence whether the intrinsic HSF1 DNA-binding activity is altered upon BGP-15 treatment.

It is unlikely that changes in nucleosome occupancy would be limited to genes regulated by HSF1. Thus, we measured MNase resistance on two HSR-unrelated genomic regions: Notch4 (Neurogenic locus notch homolog protein 4) was chosen as a signaling protein known to affect gene expression (Chitnis and Balle-Cuif, 2016), and Daxx (Death domain-associated protein) was selected as an example of a general transcriptional regulator (Lindsay *et al.*, 2008). Again, BGP-15 treatment reduced the MNase resistance across both analyzed genomic *loci* (II, Fig 3B), indicating that BGP-15 enforces changes in the chromatin structure across the genome. It would be beneficial to assess the scale of BGP-15 mediated changes in the whole genome by utilizing high-throughput sequencing techniques, such as MNase-seq or Assay for Transposase-Accessible Chromatin with high throughput sequencing (ATAC-seq)

(Buenrostro *et al.*, 2013; Cui and Zhao, 2012). The MNase-seq technique allows for genome-wide mapping of nucleosomes positioning and occupancy based on the digestion patterns generated by MNase (Cui and Zhao, 2012; Mieczkowski *et al.*, 2016). The ATAC-seq is an alternative technique to MNase-seq to measure chromatin accessibility (Buenrostro *et al.*, 2013). ATAC-seq uses Tn5 transposase to integrate Tn5 transposons into the genome. Insertion of the DNA transposons occurs preferentially in the open chromatin regions (Gangadharan *et al.*, 2010) and transposons mapping with the high-throughput sequencing allows for simultaneous interrogation of factor occupancy, nucleosome positions in regulatory sites, and chromatin accessibility in a genome-wide manner (Buenrostro *et al.*, 2013).

### 2.4. HDACs inhibitors act as chaperone co-inducers

Assuming that BGP-15 chaperone co-inducing capacity stems from its ability to inhibit HDAC activity, other HDAC inhibitors should display similar properties. We tested that hypothesis by pretreating MEFs for 1 hour with BGP-15, TSA and another well-defined HDAC inhibitor, valproic acid (VPA), (Göttlicher *et al.*, 2002), followed by exposure to heat stress at 42°C for 30 and 60 minutes. We measured the mRNA levels of *HSP70* and *DNAJB1* to evaluate the HSR. We observed that treatment of cells with TSA and VPA did not result in upregulation of HSPs under normal conditions similar to the treatment with BGP-15 (II, Fig 4A) However, upon stress, all three HDAC inhibitors were able to increase levels of HSPs. Furthermore, HSP mRNA levels in cells treated with the potent HDAC inhibitors TSA and VPA, were higher than in cells treated with BGP-15, indicating that TSA and VPA had more potent chaperone co-inducing properties. We then tested, whether enhancement of the HSR by HDAC inhibitors is capable of protecting cells from damages caused by acute stress. For this purpose, we measured the viability of the cells pretreated with HDAC inhibitors and exposed to acute stress at 42°C for 2 hours, followed by a 16-hour recovery. Enhanced HSPs expression caused by HDAC inhibitors increased cell survival both immediately after stress and during the recovery (II, Fig 4B). We also measured the cytoprotective effect of HDAC inhibitors in cells exposed to a severe heat stress, *i.e.* 45°C for 30 minutes followed by a 16-hour recovery. Again, all three HDAC inhibitors exhibited a cytoprotective effect in cells exposed to extreme stress (II, Fig 4C). Taken together, our results show that HDAC inhibition enhances the HSR and increases cell survival in the events of protein-damaging stress.

Based on our findings that BGP-15 acts as an HDAC inhibitor and increases chromatin accessibility, we propose a model where HDAC inhibition results in enhanced HSR in the event of proteotoxic insults (**Figure 14**). Since administration of BGP-15 alone is sufficient to impose changes in the chromatin structure, we propose that treatment with BGP-15 rewires chromatin to a state that is more favorable for transcription. In fact, HDAC inhibitors have been shown to increase Pol II occupancy at the paused gene (Wang *et al.*, 2009), which suggests that BGP-15 and HDAC inhibitors, in general, facilitate accelerated HSF1 binding and stress-inducible expression of HSPs.

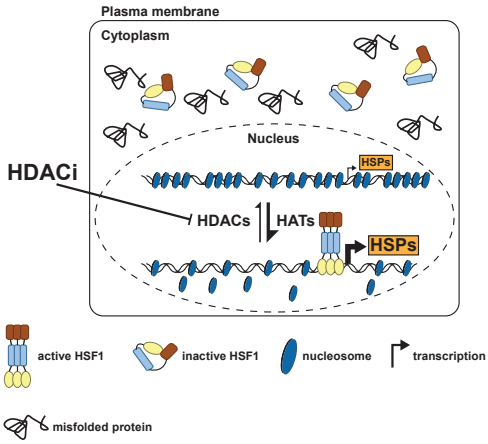


**Figure 14. A model for chaperone co-inducing effect of HDAC inhibitors.** Amount of protein damage in certain pathological conditions is not sufficient to trigger HSF1 activation and the HSR. Treatment with HDAC inhibitors (HDACi) results in chromatin opening, which lowers the threshold for HSF1 activation, allowing the inducible expression of HSPs. HATs – histone acetyltransferases, HDACs – histone deacetylases.

Interestingly, the observation that treatment with HDAC inhibitors leads to an increase in expression of HSPs has already been reported in model organisms, such as *Drosophila melanogaster* (Zhao *et al.*, 2006) and *Xenopus laevis* (Ovakim and Heikkila, 2003). In *D. melanogaster* treatment with HDAC inhibitors, TSA and sodium butyrate (BuA) increased acetylation of histones H3 and H4 during basal and stress conditions (Zhao *et al.*, 2006). Surprisingly, administration of HDAC inhibitors led to HSF1 recruitment to the *HSP70 loci* under control conditions, which, in turn, increased *HSP70* mRNA expression in the absence of stress. In addition, both TSA and BuA further increased *HSP70* expression when organisms were exposed to heat stress. In *X. laevis* embryos treatment with HDAC inhibitors TPA, VPA, and BuA did not increase *HSP70* and *HSP30* mRNA under normal conditions (Ovakim and Heikkila, 2003). Treatment of embryos with HDAC inhibitors enhanced the heat shock-induced accumulation of HSPs mRNA. These results indicate that HDAC inhibitors are able to increase HSPs expression upon stress across metazoans.

### 2.5. Pharmaceutical use of HDAC inhibitors

Elevated expression of HDACs has been reported in various forms of cancer (reviewed in West *et al.*, 2014). For example, expression of HDAC1, 5, and 7 can serve as molecular biomarkers for tumors (Ozdağ *et al.*, 2006). Overexpression of HDAC1 correlates with reduced survival of patients with lung adenocarcinoma (Minamiya *et al.*, 2011), while increased HDAC2 levels result in a poor prognosis in prostate cancer (Weichert *et al.*, 2008). HDACs promote tumorigenesis through several different mechanisms. Acetylation of tumor suppressor p53 is critical for its stability, transcriptional activity as well as p53-dependent growth arrest and apoptosis (Tang *et al.*, 2008). Deacetylation of p53 by HDACs decreases transcriptional activity of p53, which leads to upregulation of oncogenes and promotes tumor growth (Duan *et al.*, 2005). Furthermore, increased HDAC activity is linked to the transcriptional repression of tumor suppressor genes, such as CDKN1A (reviewed in Glozak and Seto, 2007). Thus, HDAC inhibitors are widely accepted as anti-cancer drugs, and some of them, such as vorinostat and romidepsin, are already approved for use in cancer patients (Olsen *et al.*, 2007; Whittaker *et al.*, 2010).

The observation that HDAC inhibitors can act as chaperone co-inducers may have an implication for the use of HDAC inhibitors as anti-cancer drugs. Given the finding that HSF1 is transcriptionally activated in many cancers (Mendillo *et al.*, 2012), further HSF1 activation by HDAC inhibitors may have negligible effects on its role in promoting tumorigenesis. However, administration of HDAC inhibitors in cases of tumors without spontaneous HSF1 activity might lead to HSF1 activation, which could severely hamper the outcome of the treatment. Hence, a combination of HDAC inhibitor therapy with HSF1 inhibitors could improve the efficacy of the treatment.

Damages to the central nervous system (CNS), such as traumatic brain injury, ischemia, and hemorrhage lead to neurological disabilities and eventually to death (reviewed in Lo *et al.*, 2003; and Rosenfeld *et al.*, 2012). Multiple studies showed that HDAC inhibitors can protect neurons from death in the event of neurobiological damage as well as contribute to improved functional recovery of neurons (reviewed in Ziemka-Nalecz and Zalewska, 2014). The neuroprotective effect of HDAC inhibitors is associated with transcriptional changes in neurons. For instance, HDAC inhibitors enhance resistance to cell death by suppression of p53-dependent apoptosis through decreasing p53 protein levels in an acetylation-mediated manner (Brochier *et al.*, 2013). Furthermore, use of HDAC inhibitors results in increased expression of many transcription factors, such as the brain-derived neurotrophic factor which regulates synaptogenesis and neurite outgrowth (Faraco *et al.*, 2006; Hasan *et al.*, 2013). The CNS damage results in upregulation of HSPs, however use of HDAC inhibitors further increases HSP levels upon damage (Lv *et al.*, 2011; Sinn *et al.*, 2007; Xuan *et al.*, 2012). HSPs are thought to act as one of the major neuroprotective proteins, due to their anti-apoptotic properties as well as *via* suppression of microglia/monocyte activation (reviewed in Yenari *et al.*, 2005). Intriguingly, VPA-mediated neuroprotection against glutamate-induced cell death was lost if HSP70 induction was blocked (Marinova *et al.*, 2009). Importantly, the increase in HSP levels that occurs even after the damage to neurons provides cytoprotection (Hoehn *et al.*, 2001). Thus, our finding that HDAC inhibitors act as co-inducers of the HSR suggests a molecular mechanism of action for their HSP-mediated cytoprotective effect on challenged neurons.

The threshold for the HSR activation in motor neurons is set much higher when compared with, for example, glial cells (Batulan *et al.*, 2003). The finding that overexpression of HSF1 in motor neurons fails to rescue expression of HSPs upon stress (Batulan *et al.*, 2003), indicates that other components than HSF1 are involved in the repression of the HSR in neurons. Our results are showing that HDAC inhibition lowers the threshold for HSF1 activation, might provide a strategy for activating HSF1-mediated expression of HSPs in pathologies, such as neurodegeneration. VPA alone has been shown to induce HSP70 levels in SH-SY5Y human neuroblastoma cells (Pan *et al.*, 2005), which supports the notion that HDAC inhibitors could increase HSP levels in neurons. However, recent studies have provided an alternative explanation for the lack of HSPs induction during the neurodegeneration. In cells expressing aggregation-prone proteins, animal models of Parkinson's and HD as well as in patient samples,

decreased levels of HSF1 protein have been observed (Gomez-Pastor *et al.*, 2017; Kim *et al.*, 2016). In HD, HSF1 phosphorylation at S303 and S307 leads to proteasomal degradation of HSF1 *via* E3 ubiquitin ligase FBXW7$\alpha$-mediated ubiquitination (Gomez-Pastor *et al.*, 2017). In Parkinson's disease, HSF1 is ubiquitinated by E3 ligase NEDD4, which in turn, results in loss of HSF1 upon $\alpha$-synuclein aggregation (Kim *et al.*, 2016). Intriguingly, HSF1 degradation by NEDD4 is dependent on the HSF1 acetylation status, since activation of SIRT1 by resveratrol prevented HSF1 degradation in the presence of $\alpha$-synuclein (Kim *et al.*, 2016). Given that HSF1 interacts with members of class I, II, and III HDACs (Westerheide *et al.*, 2009; Zelin and Freeman, 2015), and that acetylation status affects both protein levels and *trans*-activation capacity of HSF1 (Kim *et al.*, 2016; Raychaudhuri *et al.*, 2014; Westerheide *et al.*, 2009), further studies are needed to elucidate the functional outcome of HDAC inhibitors on HSF1 stability and activity in various neuropathies.

# CONCLUDING REMARKS

Since the start of my PhD thesis work, we can observe the growing interest towards understanding HSF1 and its complex role in biological processes. Multiple studies have presented evidence that HSF1 drives distinct transcriptional programs in stress, development, gametogenesis, and cancer. Yet, at the core of each of these programs, HSF1 is regulating expression of HSPs. While HSF1 is the best-studied member of the HSF family, many fundamental questions on how HSF1 and HSF1-mediated HSR are governed have remained unanswered. This thesis aimed to answer some of the longstanding questions about the regulation of the HSR. Our study, using a phosphorylation-deficient HSF1 mutant revealed that the stress-inducible phosphorylation of HSF1, in contrast to an original hypothesis, is dispensable for the HSR activation, but plays a pivotal role in fine-tuning the magnitude of the response. Furthermore, we show that the phosphorylation-deficient RD of HSF1 is still capable of sensing heat stress. Therefore, the search for understanding how the HSF1 and the HSR are activated in response to different stimuli should continue.

Pharmacological control over the HSR is perceived as an effective strategy for combating various pathologies. Activation of the HSR in neurodegenerative diseases can counteract protein aggregation. Moreover, increased HSP levels have been shown to have beneficial effects in other proteinopathies, such as muscle dystrophy or during CNS damage. Our findings that BGP-15 is an HDAC inhibitor accelerates HSF1 activation and lowers the threshold for the HSR initiation. However, it remains to be established how BGP-15 inhibits the activity of HDACs,ince since it is unable to inhibit the catalytic subunit of HDACs *in vitro*. Furthermore, our study reveals that HDAC inhibitors can function as potent chaperone co-inducers. Furthermore, we propose a molecular mechanism for HDAC inhibitors acting as chaperone co-inducers, through facilitating increasing chromatin accessibility for HSF1. These results warrant further studies that should identify which HDACs are crucial for determining chromatin landscape at the promoter region of HSP genes.

In conclusion, this thesis expands knowledge of mechanisms regulating the HSR by providing additional information on two layers involved in mounting and fine-tuning the HSR, the phosphorylation of HSF1 and chromatin landscape (**Figure 15**). Although the results reported in this thesis cannot be directly translated into medical use, they provide the basis for further development of pharmaceuticals aimed to activate, enhance or repress the HSR.

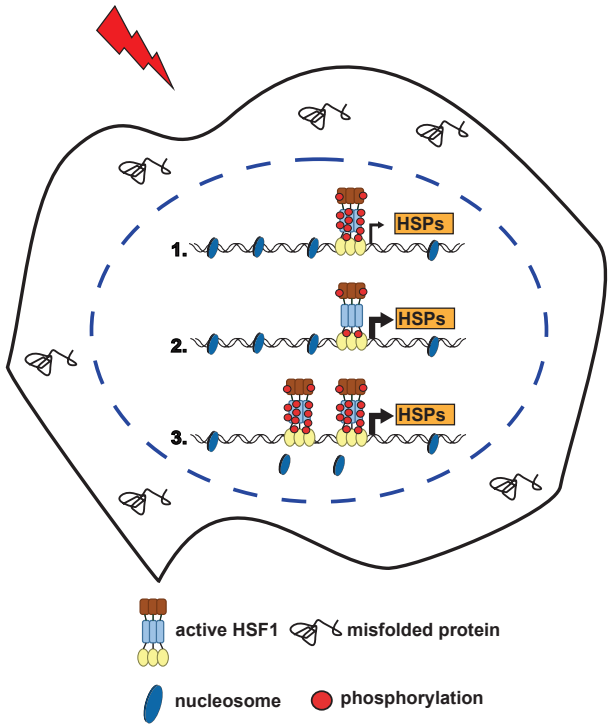


**Figure 15. Multiple layers of regulation of the HSR.** Various protein-damaging stresses result in activation of the HSR *via* HSF1-mediated transcription of heat shock proteins (HSPs). **1.** Activated HSF1 forms trimers, binds DNA, undergoes extensive post-translational modifications, such as phosphorylation, and activates expression of HSPs. **2.** While the stress-inducible phosphorylation of HSF1 is not required for HSF1 activation, phosphorylation fine-tunes the HSF1-driven response. **3.** Chromatin landscape also defines the threshold and magnitude of the HSR. For example, chromatin opening by HDACs inhibitors does not alone activate the HSR, but co-induces HSPs expression initiated by other stimuli. The dashed blue line represents the nuclear envelope.

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# REFERENCES


**Abane, R. and Mezger, V.** (2010). Roles of Heat Shock Factors in Gametogenesis and Development. *FEBS J.* **277**, 4150–4172.

**Abravaya, K., Myers, M. P., Murphy, S. P. and Morimoto, R. I.** (1992). The Human Heat Shock Protein Hsp70 Interacts with HSF, the Transcription Factor that Regulates Heat Shock Gene Expression. *Genes Dev.* **6**, 1153–1164.

**Agre, P., King, L. S., Yasui, M., Guggino, W. B., Ottersen, O. P., Fujiyoshi, Y., Engel, A. and Nielsen, S.** (2002). Aquaporin Water Channels--from Atomic Structure to Clinical Medicine. *J. Physiol.* **542**, 3–16.

**Åkerfelt, M., Henriksson, E., Laiho, A., Vihervaara, A., Rautoma, K., Kotaja, N. and Sistonen, L.** (2008). Promoter ChIP-chip Analysis in Mouse Testis Reveals Y Chromosome Occupancy by HSF2. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 11224–11229.

**Åkerfelt, M., Morimoto, R. I. and Sistonen, L.** (2010). Heat Shock Factors: Integrators of Cell Stress, Development and Lifespan. *Nat. Rev. Mol. Cell Biol.* **11**, 545–555.

**Alastalo, T.-P., Lönnström, M., Leppä, S., Kaarniranta, K., Pelto-Huikko, M., Sistonen, L. and Parvinen, M.** (1998). Stage-Specific Expression and Cellular Localization of the Heat Shock Factor 2 Isoforms in the Rat Seminiferous Epithelium. *Exp. Cell Res.* **240**, 16–27.

**Alastalo, T.-P., Hellesuo, M., Sandqvist, A., Hietakangas, V., Kallio, M. and Sistonen, L.** (2003). Formation of Nuclear Stress Granules Involves HSF2 and Coincides with the Nucleolar Localization of Hsp70. *J. Cell Sci.* **116**, 3557–3570.

**Alexander, R. P., Fang, G., Rozowsky, J., Snyder, M. and Gerstein, M. B.** (2010). Annotating Non-coding Regions of the Genome. *Nat. Rev. Genet.* **11**, 559–571.

**Allfrey, V. G., Faulkner, R. and Mirsky, A. E.** (1964). Acetylation and Methylation of Histones and their Possible Role in the Regulation of RNA Synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **51**, 786–794.

**Allis, C. D. and Jenuwein, T.** (2016). The Molecular Hallmarks of Epigenetic Control. *Nat. Rev. Genet.* **17**, 487–500.

**Amin, J., Ananthan, J. and Voellmy, R.** (1988). Key Features of Heat Shock Regulatory Elements. *Mol. Cell. Biol.* **8**, 3761–3769.

**Amin, J., Fernandez, M., Ananthan, J., Lis, J. T. and Voellmy, R.** (1994). Cooperative Binding of Heat Shock Transcription Factor to the Hsp70 Promoter in Vivo and in Vitro. *J. Biol. Chem.* **269**, 4804–4811.

**Anckar, J. and Sistonen, L.** (2011). Regulation of HSF 1 Function in the Heat Stress Response: Implications in Aging and Disease. *Annu. Rev. Biochem.* **80**, 1089–1115.

**Bah, A., Vernon, R. M., Siddiqui, Z., Krzeminski, M., Muhandiram, R., Zhao, C., Sonenberg, N., Kay, L. E. and Forman-Kay, J. D.** (2014). Folding of an Intrinsically Disordered Protein by Phosphorylation as a Regulatory Switch. *Nature* **519**, 106–109.

**Bannister, A. J. and Kouzarides, T.** (1996). The CBP Co-activator Is a Histone Acetyltransferase. *Nature* **384**, 641–643.

**Bannister, A. J. and Kouzarides, T.** (2011). Regulation of Chromatin by Histone Modifications. *Cell Res.* **21**, 381–395.

**Bannister, A. J., Zegerman, P., Partridge, J. F., Miska, E. A., Thomas, J. O., Allshire, R. C. and Kouzarides, T.** (2001). Selective Recognition of Methylated Lysine 9 on Histone H3 by the HP1 Chromo Domain. *Nature* **410**, 120–124.

**Bantscheff, M., Hopf, C., Savitski, M. M., Dittmann, A., Grandi, P., Michon, A.-M. M., Schlegl, J., Abraham, Y., Becher, I., Bergamini, G., Boesche, M., Delling, M., Dumpelfeld, B., Eberhard, D., Huthmacher, C., Mathieson, T., Poeckel, D., Reader, V., Strunk, K., et al.** (2011). Chemoproteomics Profiling of HDAC Inhibitors Reveals Selective Targeting of HDAC Complexes. *Nat. Biotechnol.* **29**, 255–265.

**Barboric, M., Nissen, R. M., Kanazawa, S., Jabrane-Ferrat, N. and Peterlin, B. M.** (2001). NF-κB Binds P-TEFb to Stimulate Transcriptional Elongation by RNA Polymerase II. *Mol. Cell* **8**, 327–337.

**Bartel, D. P.** (2004). MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. *Cell* **116**, 281–297.

**Batulan, Z., Shinder, G. A., Minotti, S., He, B. P., Doroudchi, M. M., Nalbantoglu, J., Strong, M. J. and Durham, H. D.** (2003). High Threshold for Induction of the Stress Response in Motor Neurons Is Associated with Failure to Activate HSF1. *J. Neurosci.* **23**, 5789–5798.

**Benita, Y., Kikuchi, H., Smith, A. D., Zhang, M. Q., Chung, D. C. and Xavier, R. J.** (2009). An Integrative Genomics Approach Identifies Hypoxia Inducible Factor-1 (HIF-1)-target Genes that Form the Core Response to Hypoxia. *Nucleic Acids Res.* **37**, 4587–4602.

**Benjamin, I. J., Christians, E., Davis, A. A. and Thomas, S. D.** (2000). Embryonic Development: Maternal Effect of Hsf1 Onreproductive Success. *Nature* **407**, 693–694.

**Berra, E., Roux, D., Richard, D. E. and Pouysségur, J.** (2001). Hypoxia-inducible Factor-1α (HIF-1α) Escapes O2 -driven Proteasomal Degradation Irrespective of Its Subcellular Localization: Nucleus Or Cytoplasm. *EMBO Rep.* **2**, 615–620.

**Bertolotti, A., Zhang, Y., Hendershot, L. M., Harding, H. P. and Ron, D.** (2000). Dynamic Interaction of BiP and ER Stress Transducers in the Unfolded-protein Response. *Nat. Cell Biol.* **2**, 326–332.

**Bhowmick, B. K., Takahata, N., Watanabe, M. and Satta, Y.** (2006). Comparative Analysis of Human Masculinity. *Genet. Mol. Res.* **5**, 696–712.

**Biamonti, G. and Vourc'h, C.** (2010). Nuclear Stress Bodies. *Cold Spring Harb. Perspect. Biol.* **2**, a000695.

**Bitterman, K. J., Anderson, R. M., Cohen, H. Y., Latorre-Esteves, M. and Sinclair, D. A.** (2002). Inhibition of Silencing and Accelerated Aging by Nicotinamide, A Putative Negative Regulator of Yeast Sir2 and Human SIRT1. *J. Biol. Chem.* **277**, 45099–45107.

**Boellmann, F., Guettouche, T., Guo, Y., Fenna, M., Mnayer, L. and Voellmy, R.** (2004). DAXX Interacts with Heat Shock Factor 1 During Stress Activation and Enhances Its Transcriptional Activity. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 4100–4105.

**Braakman, I. and Hebert, D. N.** (2013). Protein Folding in the Endoplasmic Reticulum. *Cold Spring Harb. Perspect. Biol.* **5**, a013201.

**Braunschweig, U., Barbosa-Morais, N. L., Pan, Q., Nachman, E. N., Alipanahi, B., Gonatopoulos-Pournatzis, T., Frey, B., Irimia, M. and Blencowe, B. J.** (2014). Widespread Intron Retention in Mammals Functionally Tunes Transcriptomes. *Genome Res.* **24**, 1774–86.

**Brochier, C., Dennis, G., Rivieccio, M. A., McLaughlin, K., Coppola, G., Ratan, R. R. and Langley, B.** (2013). Specific Acetylation of P53 by HDAC Inhibition Prevents DNA Damage-Induced Apoptosis in Neurons. *J. Neurosci.* **33**, 8621–8632.

**Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y. and Allis, C. D.** (1996). Tetrahymena Histone Acetyltransferase A: A Homolog to Yeast Gcn5p Linking Histone Acetylation to Gene Activation. *Cell* **84**, 843–851.

**Bu, L., Jin, Y., Shi, Y., Chu, R., Ban, A., Eiberg, H., Andres, L., Jiang, H., Zheng, G., Qian, M., Cui, B., Xia, Y., Liu, J., Hu, L., Zhao, G., Hayden, M. R. and Kong, X.** (2002). Mutant DNA-binding Domain of HSF4 Is Associated with Autosomal Dominant Lamellar and Marner Cataract. *Nat. Genet.* **31**, 276–278.

**Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y. and Greenleaf, W. J.** (2013). Transposition of Native Chromatin for Fast and Sensitive Epigenomic Profiling of Open Chromatin, DNA-binding Proteins and Nucleosome Position. *Nat. Methods* **10**, 1213–1218.

**Burg, M. B., Ferraris, J. D. and Dmitrieva, N. I.** (2007). Cellular Response to Hyperosmotic Stresses. *Physiol. Rev.* **87**, 1441–1474.

**Bustany, S., Cahu, J., Descamps, G., Pellat-Deceunynck, C. and Sola, B.** (2015). Heat Shock Factor 1 Is a Potent Therapeutic Target for Enhancing the Efficacy of Treatments for Multiple Myeloma with Adverse Prognosis. *J. Hematol. Oncol.* **8**, 40.

**Calfon, M., Zeng, H., Urano, F., Till, J. H., Hubbard, S. R., Harding, H. P., Clark, S. G. and Ron, D.** (2002). IRE1 Couples Endoplasmic Reticulum Load to Secretory Capacity by Processing the XBP-1 MRNA. *Nature* **415**, 92–96.

**Carmeliet, P., Dor, Y., Herbert, J.-M., Fukumura, D., Brusselmans, K., Dewerchin, M., Neeman, M., Bono, F.,**

**Abramovitch, R., Maxwell, P., Koch, C. J., Ratcliffe, P., Moons, L., Jain, R. K., Collen, D. and Keshet, E.** (1998). Role of HIF-1A in Hypoxia-mediated Apoptosis, Cell Proliferation and Tumour Angiogenesis. *Nature* **394**, 485–490.

**Chambers, A. E., Banerjee, S., Chaplin, T., Dunne, J., Debernardi, S., Joel, S. P. and Young, B. D.** (2003). Histone Acetylation-mediated Regulation of Genes in Leukaemic Cells. *Eur. J. Cancer* **39**, 1165–1175.

**Champagne, K. S. and Kutateladze, T. G.** (2009). Structural Insight into Histone Recognition by the ING PHD Fingers. *Curr. Drug Targets* **10**, 432–441.

**Chang, Y., Östling, P., Åkerfelt, M., Trouillet, D., Rallu, M., Gitton, Y., El Fatimy, R., Fardeau, V., Le Crom, S., Morange, M., Sistonen, L. and Mezger, V.** (2006). Role of Heat-shock Factor 2 in Cerebral Cortex Formation and as a Regulator of P35 Expression. *Genes Dev.* **20**, 836–847.

**Chen, Y., Chen, J., Yu, J., Yang, G., Temple, E., Harbinski, F., Gao, H., Wilson, C., Pagliarini, R. and Zhou, W.** (2014). Identification of Mixed Lineage Leukemia 1(MLL1) Protein as a Coactivator of Heat Shock Factor 1(HSF1) Protein in Response to Heat Shock Protein 90 (HSP90) Inhibition. *J. Biol. Chem.* **289**, 18914–18927.

**Chiang, W.-C., Ching, T.-T., Lee, H. C., Mousigian, C. and Hsu, A.-L.** (2012). HSF-1 Regulators DDL-1/2 Link Insulin-like Signaling to Heat-Shock Responses and Modulation of Longevity. *Cell* **148**, 322–334.

**Chitnis, A. and Balle-Cuif, L.** (2016).The Notch Meeting: An Odyssey from Structure to Function. *Development* **143**, 547–553.

**Choi, Y., Asada, S. and Uesugi, M.** (2000). Divergent HTAF(II)31-binding Motifs Hidden in Activation Domains. *J. Biol. Chem.* **275**, 15912–15916.

**Chou, S.-D., Prince, T., Gong, J. and Calderwood, S. K.** (2012). mTOR Is Essential for the Proteotoxic Stress Response, HSF1 Activation and Heat Shock Protein Synthesis. *PLoS One* **7**, e39679.

**Chu, B., Soncin, F., Price, B. D., Stevenson, M. A. and Calderwood, S. K.** (1996). Sequential Phosphorylation by Mitogen-activated Protein Kinase and Glycogen Synthase Kinase 3 Represses Transcriptional Activation by Heat Shock Factor-1. *J. Biol. Chem.* **271**, 30847–30857.

**Chu, B., Zhong, R., Soncin, F., Stevenson, M. A. and Calderwood, S. K.** (1998). Transcriptional Activity of Heat Shock Factor 1 at 37°C Is Repressed Through Phosphorylation on Two Distinct Serine Residues by Glycogen Synthase Kinase 3 and Protein Kinases C and C. *J. Biol. Chem.* **273**, 18640–18646.

**Churchman, L. S. and Weissman, J. S.** (2012). Native Elongating Transcript Sequencing (NET-seq). in *Current Protocols in Molecular Biology*, p. Unit 4.14.1-17. Hoboken, NJ, USA: John Wiley & Sons, Inc.

**Clamp, M., Fry, B., Kamal, M., Xie, X., Cuff, J., Lin, M. F., Kellis, M., Lindblad-Toh, K. and Lander, E. S.** (2007). Distinguishing Protein-coding and Noncoding Genes in the Human Genome. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 19428–19433.

**Clark, D. A., Gabel, C. V, Gabel, H. and Samuel, A. D. T.** (2007). Temporal Activity Patterns in Thermosensory Neurons of Freely Moving Caenorhabditis Elegans Encode Spatial Thermal Gradients. *J. Neurosci. Off. J. Soc. Neurosci.* **27**, 6083–6090.

**Corey, L. L., Weirich, C. S., Benjamin, I. J. and Kingston, R. E.** (2003). Localized Recruitment of a Chromatin-remodeling Activity by an Activator *In Vivo* Drives Transcriptional Elongation. *Genes Dev.* **17**, 1392–1401.

**Cox, J. S. and Walter, P.** (1996). A Novel Mechanism for Regulating Activity of a Transcription Factor that Controls the Unfolded Protein Response. *Cell* **87**, 391–404.

**Cui, K. and Zhao, K.** (2012). Genome-wide Approaches to Determining Nucleosome Occupancy in Metazoans Using MNase-Seq. *Methods Mol. Biol.* **833**, 413–419.

**Czermin, B., Melfi, R., McCabe, D., Seitz, V., Imhof, A. and Pirrotta, V.** (2002). Drosophila Enhancer of Zeste/ESC Complexes Have a Histone H3 Methyltransferase Activity that Marks Chromosomal Polycomb Sites. *Cell* **111**, 185–196.

**Dai, C., Whitesell, L., Rogers, A. B. and Lindquist, S.** (2007). Heat Shock Factor 1 Is a Powerful Multifaceted Modifier of Carcinogenesis. *Cell* **130**, 1005–1018.

**Dai, C. and Sampson, S. B.** (2016). HSF1: Guardian of Proteostasis in Cancer. *Trends Cell Biol.* **26**, 17–28.

**Dai, S., Tang, Z., Cao, J., Zhou, W., Li, H., Sampson, S. and Dai, C.** (2015). Suppression of the HSF1-mediated Proteotoxic Stress Response by the Metabolic Stress Sensor AMPK. *EMBO J.* **34**, 275–293.

**Damberger, F. F., Pelton, J. G., Harrison, C. J., Nelson, H. C. M. and Wemmer, D. E.** (1994). Solution Structure of the DNA-binding Domain of the Heat Shock Transcription Factor Determined by Multidimensional Heteronuclear Magnetic Resonance Spectroscopy. *Protein Sci.* **3**, 1806–1821.

**Das, S. and Bhattacharyya, N. P.** (2014). Heat Shock Factor 1 Regulates Hsa-miR-432 Expression in Human Cervical Cancer Cell Line. *Biochem. Biophys. Res. Commun.* **453**, 461–466.

**Davis, R. J.** (2000). Signal Transduction by the JNK Group of MAP Kinases. *Cell* **103**, 239–252.

**Dayalan, N. S. and Dinkova-Kostova, A. T.** (2017). Regulation of the Mammalian Heat Shock Factor 1. *FEBS J.* **38**, 42–49.

**Dayalan Naidu, S., Sutherland, C., Zhang, Y., Risco, A., de la Vega, L., Caunt, C. J., Hastie, C. J., Lamont, D. J., Torrente, L., Chowdhry, S., Benjamin, I. J., Keyse, S. M., Cuenda, A. and Dinkova-Kostova, A. T.** (2016). Heat Shock Factor 1 Is a Substrate for P38 Mitogen-Activated Protein Kinases. *Mol. Cell. Biol.* **36**, 2403–2417.

**Denegri, M., Moralli, D., Rocchi, M., Biggiogera, M., Raimondi, E., Cobianchi, F., De Carli, L., Riva, S. and Biamonti, G.** (2002). Human Chromosomes 9, 12, and 15 Contain the Nucleation Sites of Stress-Induced Nuclear Bodies. *Mol. Biol. Cell* **13**, 2069–2079.

**Di Giorgio, E. and Brancolini, C.** (2016). Regulation of Class IIa HDAC Activities: It Is Not only Matter of Subcellular Localization. *Epigenomics* **8**, 251–269.

**Di Lorenzo, A. and Bedford, M. T.** (2011). Histone Arginine Methylation. *FEBS Lett.* **585**, 2024–31.

**Dong, W., Zhou, Y. and Yang, Z.** (2016). Review on the Effect of Resveratrol on Central Nervous System Disease. *J. Behav. Brain Sci.* **6**, 118–123.

**Duan, H., Heckman, C. A. and Boxer, L. M.** (2005). Histone Deacetylase Inhibitors Down-Regulate Bcl-2 Expression and Induce Apoptosis in T(14;18) Lymphomas. *Mol. Cell. Biol.* **25**, 1608–1619.

**Duarte, F. M., Fuda, N. J., Mahat, D. B., Core, L. J., Guertin, M. J. and Lis, J. T.** (2016). Transcription Factors GAF and HSF Act at Distinct Regulatory Steps to Modulate Stress-induced Gene Activation. *Genes Dev.* **30**, 1731–1746.

**Dudeja, V., Chugh, R. K., Sangwan, V., Skube, S. J., Mujumdar, N. R., Antonoff, M. B., Dawra, R. K., Vickers, S. M. and Saluja, A. K.** (2011). Prosurvival Role of Heat Shock Factor 1 in the Pathogenesis of Pancreatobiliary Tumors. *AJP Gastrointest. Liver Physiol.* **300**, G948–G955.

**Eberharter, A. and Becker, P. B.** (2002). Histone Acetylation: A Switch Between Repressive and Permissive Chromatin. Second in Review on Chromatin Dynamics. *EMBO Rep.* **3**, 224–229.

**El Fatimy, R., Miozzo, F., Le Mouel, A., Abane, R., Schwendimann, L., Saberan-Djoneidi, D., de Thonel, A., Massaoudi, I., Paslaru, L., Hashimoto-Torii, K., Christians, E., Rakic, P., Gressens, P. and Mezger, V.** (2014). Heat Shock Factor 2 Is a Stress-responsive Mediator of Neuronal Migration Defects in Models of Fetal Alcohol Syndrome. *EMBO Mol. Med.* **6**, 1043–1061.

**Elsing, A. N., Aspelin, C., Björk, J. K., Bergman, H. A., Himanen, S. V., Kallio, M. J., Roos-Mattjus, P. and Sistonen, L.** (2014). Expression of HSF2 Decreases in Mitosis to Enable Stress-inducible Transcription and Cell Survival. *J. Cell Biol.* **206**, 735–749.

**Enoki, Y. and Sakurai, H.** (2011). Diversity in DNA Recognition by Heat Shock Transcription Factors (HSFs) From Model Organisms. *FEBS Lett.* **585**, 1293–1298.

**Faraco, G., Pancani, T., Formentini, L., Mascagni, P., Fossati, G., Leoni, F., Moroni, F. and Chiarugi, A.** (2006). Pharmacological Inhibition of Histone Deacetylases by Suberoylanilide Hydroxamic Acid Specifically Alters Gene Expression and Reduces Ischemic Injury in the Mouse Brain. *Mol. Pharmacol.* **70**, 1876–1884.

**Fernandes, M., Xiao, H. and Lis, J. T.** (1994). Fine Structure Analyses of the Drosophila and Saccharomyces Heat Shock Factor--Heat Shock Element Interactions. *Nucleic Acids Res.* **22**, 167–173.

**Fernandez-Medarde, A. and Santos, E.** (2011). Ras in Cancer and Developmental Diseases. *Genes Cancer* **2**, 344–358.

**Ferraris, J. D., Williams, C. K., Persaud, P., Zhang, Z., Chen, Y. and Burg, M. B.** (2002). Activity of the TonEBP/OREBP Transactivation Domain Varies Directly with Extracellular NaCl Concentration. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 739–744.

**Fiorenza, M. T., Farkas, T., Dissing, M., Kolding, D. and Zimarino, V.** (1995). Complex Expression of Murine Heat Shock Transcription Factors. *Nucleic Acids Res.* **23**, 467–474.

**Fischle, W., Dequiedt, F., Hendzel, M. J., Guenther, M. G., Lazar, M. A., Voelter, W. and Verdin, E.** (2002). Enzymatic Activity Associated with Class II HDACs Is Dependent on a Multiprotein Complex Containing HDAC3 and SMRT/N-CoR. *Mol. Cell* **9**, 45–57.

**Fritsch, M. and Wu, C.** (1999). Phosphorylation of Drosophila Heat Shock Transcription Factor. *Cell Stress Chaperones* **4**, 102–117.

**Fujikake, N., Nagai, Y., Popiel, H. A., Okamoto, Y., Yamaguchi, M. and Toda, T.** (2008). Heat Shock Transcription Factor 1-activating Compounds Suppress Polyglutamine-induced Neurodegeneration Through Induction of Multiple Molecular Chaperones. *J. Biol. Chem.* **283**, 26188–26197.

**Fujimoto, M., Izu, H., Seki, K., Fukuda, K., Nishida, T., Yamada, S., Kato, K., Yonemura, S., Inouye, S. and Nakai, A.** (2004). HSF4 Is Required for Normal Cell Growth and Differentiation During Mouse Lens Development. *EMBO J.* **23**, 4297–4306.

**Fujimoto, M., Takaki, E., Hayashi, T., Kitaura, Y., Tanaka, Y., Inouye, S. and Nakai, A.** (2005). Active HSF1 Significantly Suppresses Polyglutamine Aggregate Formation in Cellular and Mouse Models. *J. Biol. Chem.* **280**, 34908–34916.

**Fujimoto, M., Hayashida, N., Katoh, T., Oshima, K., Shinkawa, T., Prakasam, R., Tan, K., Inouye, S., Takii, R. and Nakai, A.** (2010). A Novel Mouse HSF3 Has the Potential to Activate Nonclassical Heat-Shock Genes During Heat Shock. *Mol. Biol. Cell* **21**, 106–116.

**Fujimoto, M., Takaki, E., Takii, R., Tan, K., Prakasam, R., Hayashida, N., Iemura, S., Natsume, T. and Nakai, A.** (2012). RPA Assists HSF1 Access to Nucleosomal DNA by Recruiting Histone Chaperone FACT. *Mol. Cell* **48**, 182–194.

**Fulda, S., Gorman, A. M., Hori, O. and Samali, A.** (2010). Cellular Stress Responses: Cell Survival and Cell Death. *Int. J. Cell Biol.* **2010**, 1–23.

**Gallo, G. J., Prentice, H. and Kingston, R. E.** (1993). Heat Shock Factor Is Required for Growth at Normal Temperatures in the Fission Yeast Schizosaccharomyces Pombe. *Mol. Cell. Biol.* **13**, 749–761.

**Gangadharan, S., Mularoni, L., Fain-Thornton, J., Wheelan, S. J. and Craig, N. L.** (2010). DNA Transposon Hermes Inserts Into DNA in Nucleosome-free Regions *In Vivo*. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 21966–21972.

**Gao, L., Cueto, M. A., Asselbergs, F. and Atadja, P.** (2002). Cloning and Functional Characterization of HDAC11, a Novel Member of the Human Histone Deacetylase Family. *J. Biol. Chem.* **277**, 25748–25755.

**Gasiunas, G., Barrangou, R., Horvath, P. and Siksnys, V.** (2012). Cas9-crRNA Ribonucleoprotein Complex Mediates Specific DNA Cleavage for Adaptive Immunity in Bacteria. *Proc. Natl. Acad. Sci. U. S. A.* **109**, E2579-86.

**Gehrig, S. M., van der Poel, C., Sayer, T. A., Schertzer, J. D., Henstridge, D. C., Church, J. E., Lamon, S., Russell, A. P., Davies, K. E., Febbraio, M. A. and Lynch, G. S.** (2012). Hsp72 Preserves Muscle Function and Slows Progression of Severe Muscular Dystrophy. *Nature* **484**, 394–398.

**Gerstein, M. B., Bruce, C., Rozowsky, J. S., Zheng, D., Du, J., Korbel, J. O., Emanuelsson, O., Zhang, Z. D., Weissman, S. and Snyder, M.** (2007). What Is A Gene, Post-ENCODE? History and Updated Definition. *Genome Res.* **17**, 669–681.

**Glaser, K. B., Staver, M. J., Waring, J. F., Stender, J., Ulrich, R. G. and Davidsen, S. K.** (2003). Gene Expression Profiling of Multiple Histone Deacetylase (HDAC) Inhibitors: Defining a Common Gene Set Produced by HDAC Inhibition in T24 and MDA Carcinoma Cell Lines. *Mol. Cancer Ther.* **2**, 151–163.

**Glozak, M. A. and Seto, E.** (2007). Histone Deacetylases and Cancer. *Oncogene* **26**, 5420–5432.

**Goenka, A., Sengupta, S., Pandey, R., Parihar, R., Mohanta, G. C., Mukerji, M. and Ganesh, S.** (2016). Human Satellite-III Non-coding RNAs Modulate Heat-shock-induced Transcriptional Repression. *J. Cell Sci.* **129**,

3541–3552.

**Goldberg, M.** (1979). Sequence Analysis of Drosophila Histone Genes. PhD Thesis, Stanford University.

**Gombos, I., Crul, T., Piotto, S., Güngör, B., Török, Z., Balogh, G., Péter, M., Slotte, J. P., Campana, F., Pilbat, A.-M., Hunya, Á., Tóth, N., Literati-Nagy, Z., Vígh, L., Glatz, A., Brameshuber, M., Schütz, G. J., Hevener, A., Febbraio, M. A., et al.** (2011). Membrane-lipid Therapy in Operation: the HSP Co-inducer BGP-15 Activates Stress Signal Transduction Pathways by Remodeling Plasma Membrane Rafts. *PLoS One* **6**, e28818.

**Gómez, A. V., Galleguillos, D., Maass, J. C., Battaglioli, E., Kukuljan, M. and Andrés, M. E.** (2008). CoREST Represses the Heat Shock Response Mediated by HSF1. *Mol. Cell* **31**, 222–231.

**Gomez-Pastor, R., Burchfiel, E. T., Neef, D. W., Jaeger, A. M., Cabiscol, E., McKinstry, S. U., Doss, A., Aballay, A., Lo, D. C., Akimov, S. S., Ross, C. A., Eroglu, C. and Thiele, D. J.** (2017). Abnormal Degradation of the Neuronal Stress-protective Transcription Factor HSF1 in Huntington's Disease. *Nat. Commun.* **8**, 14405.

**Goodrich, J. A. and Tjian, R.** (1994). TBP-TAF Complexes: Selectivity Factors for Eukaryotic Transcription. *Curr. Opin. Cell Biol.* **6**, 403–409.

**Goodson, M. L. and Sarge, K. D.** (1995). Heat-inducible DNA Binding of Purified Heat Shock Transcription Factor 1. *J. Biol. Chem.* **270**, 2447–2450.

**Göttlicher, M., Minucci, S., Zhu, P., Krämer, O. H., Schimpf, A., Giavara, S., Sleeman, J. P., Lo Coco, F., Nervi, C., Pelicci, P. G. and Heinzel, T.** (2002). Valproic Acid Defines A Novel Class of HDAC Inhibitors Inducing Differentiation of Transformed Cells. *EMBO J.* **20**, 6969–6978.

**Gómez, A. V., Galleguillos, D., Maass, J. C., Battaglioli, E., Kukuljan, M. and Andrés, M. E.** (2008). CoREST Represses the Heat Shock Response Mediated by HSF1. *Mol. Cell* **31**, 222–231.

**Green, M., Schuetz, T. J., Sullivan, E. K. and Kingston, R. E.** (1995). A Heat Shock-responsive Domain of Human HSF1 that Regulates Transcription Activation Domain Function. *Mol. Cell. Biol.* **15**, 3354–3362.

**Gromiha, M. M.** (2007). Prediction of Protein Stability Upon Point Mutations. *Biochem. Soc. Trans.* **35**, 1569–73.

**Grozinger, C. M. and Schreiber, S. L.** (2000). Regulation of Histone Deacetylase 4 and 5 and Transcriptional Activity by 14-3- 3-dependent Cellular Localization. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7835–7840.

**Guenther, M. G., Levine, S. S., Boyer, L. A., Jaenisch, R. and Young, R. A.** (2007). A Chromatin Landmark and Transcription Initiation At Most Promoters in Human Cells. *Cell* **130**, 77–88.

**Guertin, M. J. and Lis, J. T.** (2010). Chromatin Landscape Dictates HSF Binding to Target DNA Elements. *PLoS Genet.* **6**, e1001114.

**Guettouche, T., Boellmann, F., Lane, W. S. and Voellmy, R.** (2005). Analysis of Phosphorylation of Human Heat Shock Factor 1 in Cells Experiencing A Stress. *BMC Biochem.* **6**, 4.

**Guisbert, E., Czyz, D. M., Richter, K., McMullen, P. D. and Morimoto, R. I.** (2013). Identification of a Tissue-Selective Heat Shock Response Regulatory Network. *PLoS Genet.* **9**, e1003466.

**Harding, H. P., Zhang, Y. and Ron, D.** (1999). Protein Translation and Folding Are Coupled by an Endoplasmic-reticulum-resident Kinase. *Nature* **397**, 271–274.

**Hargitai, J., Lewis, H., Boros, I., Rácz, T., Fiser, A., Kurucz, I., Benjamin, I., Vígh, L., Pénzes, Z., Csermely, P. and Latchman, D. S.** (2003). Bimoclomol, a Heat Shock Protein Co-inducer, Acts by the Prolonged Activation of Heat Shock Factor-1. *Biochem. Biophys. Res. Commun.* **307**, 689–695.

**Harrison, C., Bohm, A. and Nelson, H.** (1994). Crystal Structure of the DNA Binding Domain of the Heat Shock Transcription Factor. *Science* **263**, 224–227.

**Hartl, F. U. and Hayer-Hartl, M.** (2002). Molecular Chaperones in the Cytosol: From Nascent Chain to Folded Protein. *Science* **295**, 1852–1858.

**Hartl, F. U., Bracher, A. and Hayer-Hartl, M.** (2011). Molecular Chaperones in Protein Folding and Proteostasis. *Nature* **475**, 324–332.

**Hasan, M. R., Kim, J.-H., Kim, Y. J., Kwon, K. J., Shin, C. Y., Kim, H. Y., Han, S.-H., Choi, D.-H. and Lee, J.** (2013). Effect of HDAC Inhibitors on Neuroprotection and Neurite Outgrowth in Primary Rat Cortical

Neurons Following Ischemic Insult. *Neurochem. Res.* **38**, 1921–1934.

**Heimberger, T., Andrulis, M., Riedel, S., Stühmer, T., Schraud, H., Beilhack, A., Bumm, T., Bogen, B., Einsele, H., Bargou, R. C. and Chatterjee, M.** (2013). The Heat Shock Transcription Factor 1 as a Potential New Therapeutic Target in Multiple Myeloma. *Br. J. Haematol.* **160**, 465–476.

**Hendriks, I. A., Lyon, D., Young, C., Jensen, L. J., Vertegaal, A. C. O. and Nielsen, M. L.** (2017). Site-specific Mapping of the Human SUMO Proteome Reveals Co-modification with Phosphorylation. *Nat. Struct. Mol. Biol.* **24**, 325–336.

**Henstridge, D. C., Bruce, C. R., Drew, B. G., Tory, K., Kolonics, A., Estevez, E., Chung, J., Watson, N., Gardner, T., Lee-Young, R. S., Connor, T., Watt, M. J., Carpenter, K., Hargreaves, M., McGee, S. L., Hevener, A. L. and Febbraio, M. A.** (2014). Activating HSP72 in Rodent Skeletal Muscle Increases Mitochondrial Number and Oxidative Capacity and Decreases Insulin Resistance. *Diabetes* **63**, 1881–1894.

**Hentze, N., Breton, L. Le, Wiesner, J., Kempf, G., Mayer, M. P., Le Breton, L., Wiesner, J., Kempf, G. and Mayer, M. P.** (2016). Molecular Mechanism of Thermosensory Function of Human Heat Shock Transcription Factor Hsf1. *Elife* **5**, 1–24.

**Hietakangas, V., Ahlskog, J. K., Jakobsson, A. M., Hellesuo, M., Sahlberg, N. M., Holmberg, C. I., Mikhailov, A., Palvimo, J. J., Pirkkala, L. and Sistonen, L.** (2003). Phosphorylation of Serine 303 Is a Prerequisite for the Stress-Inducible SUMO Modification of Heat Shock Factor 1. *Mol. Cell. Biol.* **23**, 2953–2968.

**Hietakangas, V., Anckar, J., Blomster, H. A., Fujimoto, M., Palvimo, J. J., Nakai, A. and Sistonen, L.** (2006). PDSM, a Motif for Phosphorylation-dependent SUMO Modification. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 45–50.

**Hitomi, J., Katayama, T., Eguchi, Y., Kudo, T., Taniguchi, M., Koyama, Y., Manabe, T., Yamagishi, S., Bando, Y., Imaizumi, K., Tsujimoto, Y. and Tohyama, M.** (2004). Involvement of Caspase-4 in Endoplasmic Reticulum Stress-induced Apoptosis and Abeta-induced Cell Death. *J. Cell Biol.* **165**, 347–56.

**Hoehn, B., Ringer, T. M., Xu, L., Giffard, R. G., Sapolsky, R. M., Steinberg, G. K. and Yenari, M. A.** (2001). Overexpression of HSP72 After Induction of Experimental Stroke Protects Neurons From Ischemic Damage. *J. Cereb. Blood Flow Metab.* **21**, 1303–1309.

**Holmberg, C. I., Leppä, S., Eriksson, J. E. and Sistonen, L.** (1997). The Phorbol Ester 12-O-tetradecanoylphorbol 13-acetate Enhances the Heat-induced Stress Response. *J. Biol. Chem.* **272**, 6792–6798.

**Holmberg, C. I., Illman, S. A., Kallio, M., Mikhailov, A. and Sistonen, L.** (2000). Formation of Nuclear HSF1 Granules Varies Depending on Stress Stimuli. *Cell Stress Chaperones* **5**, 219–228.

**Holmberg, C. I., Hietakangas, V., Mikhailov, A., Rantanen, J. O., Kallio, M., Meinander, A., Hellman, J., Morrice, N., MacKintosh, C., Morimoto, R. I., Eriksson, J. E. and Sistonen, L.** (2001). Phosphorylation of Serine 230 Promotes Inducible Transcriptional Activity of Heat Shock Factor 1. *EMBO J.* **20**, 3800–3810.

**Hong, S., Kim, S. H., Heo, M. A., Choi, Y. H., Park, M. J., Yoo, M. A., Kim, H. Do, Kang, H. S. and Cheong, J.** (2004). Coactivator ASC-2 Mediates Heat Shock Factor 1-mediated Transactivation Dependent on Heat Shock. *FEBS Lett.* **559**, 165–170.

**Howitz, K. T., Bitterman, K. J., Cohen, H. Y., Lamming, D. W., Lavu, S., Wood, J. G., Zipkin, R. E., Chung, P., Kisielewski, A., Zhang, L.-L., Scherer, B. and Sinclair, D. A.** (2003). Small Molecule Activators of Sirtuins Extend Saccharomyces Cerevisiae Lifespan. *Nature* **425**, 191–196.

**Hsin, J.-P. and Manley, J. L.** (2012). The RNA Polymerase II CTD Coordinates Transcription and RNA Processing. *Genes Dev.* **26**, 2119–2137.

**Hsu, A.-L., Murphy, C. T. and Kenyon, C.** (2003). Regulation of Aging and Age-Related Disease by DAF-16 and Heat-Shock Factor. *Science* **300**, 1142–1145.

**Huang, M., Li, D., Huang, Y., Cui, X., Liao, S., Wang, J., Liu, F., Li, C., Gao, M., Chen, J., Tang, Z., Li, D. W. C. and Liu, M.** (2015). HSF4 Promotes G1/S Arrest in Human Lens Epithelial Cells by Stabilizing P53. *Biochim. Biophys. Acta - Mol. Cell Res.* **1853**, 1808–1817.

**Hundley, H., Eisenman, H., Walter, W., Evans, T., Hotokezaka, Y., Wiedmann, M. and Craig, E.** (2002). The *In Vivo* Function of the Ribosome-associated Hsp70, Ssz1, does Not Require Its Putative Peptide-binding Domain. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 4203–4208.

**Hussong, M., Kaehler, C., Kerick, M., Grimm, C., Franz, A., Timmermann, B., Welzel, F., Isensee, J., Hucho, T., Krobitsch, S. and Schweiger, M. R.** (2016). The Bromodomain Protein BRD4 Regulates Splicing During Heat Shock. *Nucleic Acids Res.* 1–13.

**Imazu, H. and Sakurai, H.** (2005). Saccharomyces Cerevisiae Heat Shock Transcription Factor Regulates Cell Wall Remodeling in Response to Heat Shock. *Eukaryot. Cell* **4**, 1050–1056.

**Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., Oyake, T., Hayashi, N., Satoh, K., Hatayama, I., Yamamoto, M. and Nabeshima, Y.** (1997). An Nrf2/small Maf Heterodimer Mediates the Induction of Phase II Detoxifying Enzyme Genes Through Antioxidant Response Elements. *Biochem. Biophys. Res. Commun.* **236**, 313–322.

**Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., Salic, A., Asara, J. M., Lane, W. S. and Kaelin, W. G.** (2001). HIFalpha Targeted for VHL-mediated Destruction by Proline Hydroxylation: Implications for $O_2$ Sensing. *Science* **292**, 464–468.

**Jaakkola, P., Mole, D. R., Tian, Y. M., Wilson, M. I., Gielbert, J., Gaskell, S. J., von Kriegsheim, A., Hebestreit, H. F., Mukherji, M., Schofield, C. J., Maxwell, P. H., Pugh, C. W. and Ratcliffe, P. J.** (2001). Targeting of HIF-alpha to the Von Hippel-Lindau Ubiquitylation Complex by $O_2$-regulated Prolyl Hydroxylation. *Science* **292**, 468–472.

**Jaeger, A. M., Makley, L. N., Gestwicki, J. E. and Thiele, D. J.** (2014). Genomic Heat Shock Element Sequences Drive Cooperative Human Heat Shock Factor 1 DNA Binding and Selectivity. *J. Biol. Chem.* **289**, 30459–30469.

**Jaeger, A. M., Pemble, C. W., Sistonen, L. and Thiele, D. J.** (2016). Structures of HSF2 Reveal Mechanisms for Differential Regulation of Human Heat-shock Factors. *Nat. Struct. Mol. Biol.* **23**, 147–154.

**Jakobsen, B. K. and Pelham, H. R.** (1991). A Conserved Heptapeptide Restrains the Activity of the Yeast Heat Shock Transcription Factor. *EMBO J.* **10**, 369–375.

**Jing, Z., Gangalum, R. K., Bhat, A. M., Nagaoka, Y., Jiang, M. and Bhat, S. P.** (2014). HSF4 Mutation P.Arg116His Found in Age-Related Cataracts and in Normal Populations Produces Childhood Lamellar Cataract in Transgenic Mice. *Hum. Mutat.* **35**, 1068–1071.

**Johnson, L. N. and Barford, D.** (1993). The Effects of Phosphorylation on the Structure and Function of Proteins. *Annu. Rev. Biophys. Biomol. Struct.* **22**, 199–232.

**Jolly, C., Metz, A., Govin, J., Vigneron, M., Turner, B. M., Khochbin, S. and Vourc'h, C.** (2004). Stress-induced Transcription of Satellite III Repeats. *J. Cell Biol.* **164**, 25–33.

**Jonkers, I., Kwak, H. and Lis, J. T.** (2014). Promoter-proximal Pausing of RNA Polymerase II: Emerging Roles in Metazoans. *Elife* **3**, 1–25.

**Jurivich, D., Sistonen, L., Kroes, R. and Morimoto, R.** (1992). Effect of Sodium Salicylate on the Human Heat Shock Response. *Science* **255**, 1243–1245.

**Jurivich, D. A., Pachetti, C., Qiu, L. and Welk, J. F.** (1995). Salicylate Triggers Heat Shock Factor Differently than Heat. *J. Biol. Chem.* **270**, 24489–24495.

**Kadonaga, J. T.** (2012). Perspectives on the RNA Polymerase II Core Promoter. *Wiley Interdiscip. Rev. Dev. Biol.* **1**, 40–51.

**Kakkar, V., Meister-Broekema, M., Minoia, M., Carra, S. and Kampinga, H. H.** (2014). Barcoding Heat Shock Proteins to Human Diseases: Looking Beyond the Heat Shock Response. *Dis. Model. Mech.* **7**, 421–34.

**Kallio, M., Chang, Y., Manuel, M., Alastalo, T. P., Rallu, M., Gitton, Y., Pirkkala, L., Loones, M. T., Paslaru, L., Larney, S., Hiard, S., Morange, M., Sistonen, L. and Mezger, V.** (2002). Brain Abnormalities, Defective Meiotic Chromosome Synapsis and Female Subfertility in HSF2 Null Mice. *EMBO J.* **21**, 2591–2601.

**Kampinga, H. H., Hageman, J., Vos, M. J., Kubota, H., Tanguay, R. M., Bruford, E. A., Cheetham, M. E., Chen, B. and Hightower, L. E.** (2009). Guidelines for the Nomenclature of the Human Heat Shock Proteins. *Cell Stress Chaperones* **14**, 105–111.

**Kampinga, H. H. and Craig, E. A.** (2010). The HSP70 Chaperone Machinery: J Proteins as Drivers of Functional Specificity. *Nat. Rev. Mol. Cell Biol.* **11**, 579–592.

**Kampinga, H. H. and Bergink, S.** (2016). Heat Shock Proteins as Potential Targets for Protective Strategies in Neurodegeneration. *Lancet Neurol.* **15**, 748–759.

**Katsuno, M., Sang, C., Adachi, H., Minamiyama, M., Waza, M., Tanaka, F., Doyu, M. and Sobue, G.** (2005). Pharmacological Induction of Heat-shock Proteins Alleviates Polyglutamine-mediated Motor Neuron Disease. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 16801–16806.

**Keegan, L., Gill, G. and Ptashne, M.** (1986). Separation of DNA Binding From the Transcription-activating Function of A Eukaryotic Regulatory Protein. *Science* **231**, 699–704.

**Kieran, D., Kalmar, B., Dick, J. R. T., Riddoch-Contreras, J., Burnstock, G. and Greensmith, L.** (2004). Treatment with Arimoclomol, a Coinducer of Heat Shock Proteins, Delays Disease Progression in ALS Mice. *Nat. Med.* **10**, 402–405.

**Kim, S.-A., Yoon, J.-H., Lee, S.-H. and Ahn, S.-G.** (2005a). Polo-like Kinase 1 Phosphorylates Heat Shock Transcription Factor 1 and Mediates Its Nuclear Translocation During Heat Stress. *J. Biol. Chem.* **280**, 12653–12657.

**Kim, T. H., Barrera, L. O., Zheng, M., Qu, C., Singer, M. A., Richmond, T. A., Wu, Y., Green, R. D. and Ren, B.** (2005b). A High-resolution Map of Active Promoters in the Human Genome. *Nature* **436**, 876–880.

**Kim, J., Daniel, J., Espejo, A., Lake, A., Krishna, M., Xia, L., Zhang, Y. and Bedford, M. T.** (2006). Tudor, MBT and Chromo Domains Gauge the Degree of Lysine Methylation. *EMBO Rep* **7**, 397–403.

**Kim, J., Guermah, M., McGinty, R. K., Lee, J.-S., Tang, Z., Milne, T. A., Shilatifard, A., Muir, T. W. and Roeder, R. G.** (2009). RAD6-Mediated Transcription-Coupled H2B Ubiquitylation Directly Stimulates H3K4 Methylation in Human Cells. *Cell* **137**, 459–471.

**Kim, E., Wang, B., Sastry, N., Masliah, E., Nelson, P. T., Cai, H. and Liao, F. F.** (2016). NEDD4-mediated HSF1 Degradation Underlies $\alpha$-synucleinopathy. *Hum. Mol. Genet.* **25**, 211–222.

**Kinoshita, K., Shinka, T., Sato, Y., Kurahashi, H., Kowa, H., Chen, G., Umeno, M., Toida, K., Kiyokage, E., Nakano, T., Ito, S. and Nakahori, Y.** (2006). Expression Analysis of a Mouse Orthologue of HSFY, a Candidate for the Azoospermic Factor on the Human Y Chromosome. *J. Med. Investig.* **53**, 117–122.

**Kirkman, H. N., Rolfo, M., Ferraris, A. M. and Gaetani, G. F.** (1999). Mechanisms of Protection of Catalase by NADPH. Kinetics and Stoichiometry. *J. Biol. Chem.* **274**, 13908–13914.

**Kline, M. P. and Morimoto, R. I.** (1997). Repression of the Heat Shock Factor 1 Transcriptional Activation Domain Is Modulated by Constitutive Phosphorylation. *Mol. Cell. Biol.* **17**, 2107–2115.

**Knauf, U., Newton, E. M., Kyriakis, J. and Kingston, R. E.** (1996). Repression of Human Heat Shock Factor 1 Activity at Control Temperature by Phosphorylation. *Genes Dev.* **10**, 2782–2793.

**Ko, B. C. B., Turck, C. W., Lee, K. W. Y., Yang, Y. and Chung, S. S. M.** (2000). Purification, Identification, and Characterization of an Osmotic Response Element Binding Protein. *Biochem. Biophys. Res. Commun.* **270**, 52–61.

**Komarnitsky, P. B., Michel, B. and Buratowski, S.** (1999). TFIID-specific Yeast TAF40 Is Essential for the Majority of RNA Polymerase II-mediated Transcription *In Vivo*. *Genes Dev.* **13**, 2484–2489.

**Korfanty, J., Stokowy, T., Widlak, P., Gogler-Piglowska, A., Handschuh, L., Podkowiński, J., Vydra, N., Naumowicz, A., Toma-Jonik, A. and Widlak, W.** (2014). Crosstalk Between HSF1 and HSF2 During the Heat Shock Response in Mouse Testes. *Int. J. Biochem. Cell Biol.* **57**, 76–83.

**Kourtis, N., Moubarak, R. S., Aranda-Orgilles, B., Lui, K., Aydin, I. T., Trimarchi, T., Darvishian, F., Salvaggio, C., Zhong, J., Bhatt, K., Chen, E. I., Celebi, J. T., Lazaris, C., Tsirigos, A., Osman, I., Hernando, E. and Aifantis, I.** (2015). FBXW7 Modulates Cellular Stress Response and Metastatic Potential Through HSF1 Post-translational Modification. *Nat. Cell Biol.* **17**, 322–332.

**Krogan, N. J., Dover, J., Khorrami, S., Greenblatt, J. F., Schneider, J., Johnston, M. and Shilatifard, A.** (2002). COMPASS, a Histone H3 (Lysine 4) Methyltransferase Required for Telomeric Silencing of Gene Expression. *J. Biol. Chem.* **277**, 10753–10755.

**Kuzmichev, A., Nishioka, K., Erdjument-Bromage, H., Tempst, P. and Reinberg, D.** (2002). Histone Methyltransferase Activity Associated with a Human Multiprotein Complex Containing the Enhancer of

Zeste Protein. *Genes Dev.* **16**, 2893–2905.

**Kwak, H. and Lis, J. T.** (2013). Control of Transcriptional Elongation. *Annu. Rev. Genet.* **47**, 483–508.

**Kwak, H., Fuda, N. J., Core, L. J. and Lis, J. T.** (2013). Precise Maps of RNA Polymerase Reveal How Promoters Direct Initiation and Pausing. *Science* **339**, 950–953.

**Labbadia, J. and Morimoto, R. I. I.** (2015). Repression of the Heat Shock Response Is A Programmed Event At the Onset of Reproduction. *Mol. Cell* **59**, 639–650.

**Labbadia, J., Cunliffe, H., Weiss, A., Katsyuba, E., Sathasivam, K., Seredenina, T., Woodman, B., Moussaoui, S., Frentzel, S., Luthi-Carter, R., Paganetti, P. and Bates, G. P.** (2011). Altered Chromatin Architecture Underlies Progressive Impairment of the Heat Shock Response in Mouse Models of Huntington Disease. *J. Clin. Invest.* **121**, 3306–3319.

**Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. and Jenuwein, T.** (2001). Methylation of Histone H3 Lysine 9 Creates a Binding Site for HP1 Proteins. *Nature* **410**, 116–120.

**Laplante, M. and Sabatini, D. M.** (2012). MTOR Signaling in Growth Control and Disease. *Cell* **149**, 274–293.

**Larson, J. S., Schuetz, T. J. and Kingston, R. E.** (1995). *In Vitro* Activation of Purified Human Heat Shock Factor by Heat. *Biochemistry* **34**, 1902–1911.

**Lau, P. N. I. and Cheung, P.** (2011). Histone Code Pathway Involving H3 S28 Phosphorylation and K27 Acetylation Activates Transcription and Antagonizes Polycomb Silencing. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 2801–2806.

**Laurila, K. and Vihinen, M.** (2009). Prediction of Disease-related Mutations Affecting Protein Localization. *BMC Genomics* **10**, 122.

**Leach, M. D., Farrer, R. A., Tan, K., Miao, Z., Walker, L. A., Cuomo, C. A., Wheeler, R. T., Brown, A. J. P., Wong, K. H. and Cowen, L. E.** (2016). Hsf1 and Hsp90 Orchestrate Temperature-dependent Global Transcriptional Remodelling and Chromatin Architecture in Candida Albicans. *Nat. Commun.* **7**, 11704.

**Lee, A.-H., Iwakoshi, N. N. and Glimcher, L. H.** (2003). XBP-1 Regulates a Subset of Endoplasmic Reticulum Resident Chaperone Genes in the Unfolded Protein Response. *Mol. Cell. Biol.* **23**, 7448–7459.

**Lee, J.-S., Shukla, A., Schneider, J., Swanson, S. K., Washburn, M. P., Florens, L., Bhaumik, S. R. and Shilatifard, A.** (2007). Histone Crosstalk Between H2B Monoubiquitination and H3 Methylation Mediated by COMPASS. *Cell* **131**, 1084–1096.

**Lee, Y. J., Kim, E. H., Jae, S. L., Jeoung, D., Bae, S., Seung, H. K. and Lee, Y. S.** (2008). HSF1 as a Mitotic Regulator: Phosphorylation of HSF1 by Plk1 Is Essential for Mitotic Progression. *Cancer Res.* **68**, 7550–7560.

**Lee, S. D., Choi, S. Y., Lim, S. W., Lamitina, S. T., Ho, S. N., Go, W. Y. and Kwon, H. M.** (2011). TonEBP Stimulates Multiple Cellular Pathways for Adaptation to Hypertonic Stress: Organic Osmolyte-dependent and -independent Pathways. *AJP Ren. Physiol.* **300**, F707–F715.

**Li, Y., Xu, D., Bao, C., Zhang, Y., Chen, D., Zhao, F., Ding, J., Liang, L., Wang, Q., Liu, L., Li, J., Yao, M., Huang, S. and He, X.** (2015). MicroRNA-135b, a HSF1 Target, Promotes Tumor Invasion and Metastasis by Regulating RECK and EVI5 in Hepatocellular Carcinoma. *Oncotarget* **6**, 2421–2433.

**Li, J., Chauve, L., Phelps, G., Brielmann, R. M. and Morimoto, R. I.** (2016). E2F Coregulates an Essential HSF Developmental Program that Is Distinct From the Heat-shock Response. *Genes Dev.* **30**, 1–14.

**Lianos, G. D., Alexiou, G. A., Mangano, A. A., Mangano, A. A., Rausei, S., Boni, L., Dionigi, G. and Roukos, D. H.** (2015). The Role of Heat Shock Proteins in Cancer. *Cancer Lett.* **360**, 114–118.

**Lindquist, S.** (1986). The Heat-Shock Response. *Annu. Rev. Biochem.* **55**, 1151–1191.

**Lindsay, C. R., Morozov, V. M. and Ishov, A. M.** (2008). PML NBs (ND10) and Daxx: From Nuclear Structure to Protein Function. *Front. Biosci.* **13**, 7132–7142.

**Lis, J. T., Mason, P., Peng, J., Price, D. H. and Werner, J.** (2000). P-TEFb Kinase Recruitment and Function at Heat Shock Loci. *Genes Dev.* **14**, 792–803.

**Literáti-Nagy, B., Kulcsár, E., Literáti-Nagy, Z., Buday, B., Péterfai, É., Horváth, T., Tory, K., Kolonics, A., Fleming, A., Mandl, J. and Korányi, L.** (2009). Improvement of Insulin Sensitivity by a Novel Drug, BGP-

15, in Insulin-resistant Patients: A Proof of Concept Randomized Double-blind Clinical Trial. *Horm. Metab. Res.* **41**, 374–380.

**Literáti-Nagy, B., Péterfai, É., Kulcsár, E., Literáti-Nagy, Z., Buday, B., Tory, K., Mandl, J., Sümegi, B., Fleming, A., Roth, J. and Korányi, L.** (2010). Beneficial Effect of the Insulin Sensitizer (HSP Inducer) BGP-15 on Olanzapine-induced Metabolic Disorders. *Brain Res. Bull.* **83**, 340–344.

**Literáti-Nagy, Z., Tory, K., Literáti-Nagy, B., Kolonics, A., Török, Z., Gombos, I., Balogh, G., Vígh, L., Horváth, I., Mandl, J., Sümegi, B., Hooper, P. L. and Vígh, L.** (2012). The HSP Co-inducer BGP-15 Can Prevent the Metabolic Side Effects of the Atypical Antipsychotics. *Cell Stress Chaperones* **17**, 517–521.

**Littlefield, O. and Nelson, H. C. M.** (1999). A New Use for the "Wing" of the "Winged" Helix-turn-helix Motif in the HSF-DNA Cocrystal. *Nat. Struct. Biol.* **6**, 464–470.

**Liu, X.-D., Liu, P. C., Santoro, N. and Thiele, D. J.** (1997). Conservation of a Stress Response: Human Heat Shock Transcription Factors Functionally Substitute for Yeast HSF. *EMBO J.* **16**, 6466–6477.

**Liu, C. Y., Xu, Z. and Kaufman, R. J.** (2003). Structure and Intermolecular Interactions of the Luminal Dimerization Domain of Human IRE1alpha. *J. Biol. Chem.* **278**, 17680–17687.

**Liu, F., Pore, N., Kim, M., Voong, K. R., Dowling, M., Maity, A. and Kao, G. D.** (2006). Regulation of Histone Deacetylase 4 Expression by the SP Family of Transcription Factors. *Mol. Biol. Cell* **17**, 585–597.

**Lo, W. S., Trievel, R. C., Rojas, J. R., Duggan, L., Hsu, J. Y., Allis, C. D., Marmorstein, R. and Berger, S. L.** (2000). Phosphorylation of Serine 10 in Histone H3 Is Functionally Linked *In Vitro* and *In Vivo* to Gcn5-mediated Acetylation at Lysine 14. *Mol. Cell* **5**, 917–926.

**Lo, E. H., Dalkara, T. and Moskowitz, M. A.** (2003). Neurological Diseases: Mechanisms, Challenges and Opportunities in Stroke. *Nat. Rev. Neurosci.* **4**, 399–414.

**Loew, O.** (1900). A New Enzyme of General Occurrence in Organismis. *Science* **11**, 701–702.

**Lopez-Rodríguez, C., Aramburu, J., Rakeman, A. S. and Rao, A.** (1999). NFAT5, a Constitutively Nuclear NFAT Protein that Does Not Cooperate with Fos and Jun. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 7214–7219.

**Lu, P. D., Harding, H. P. and Ron, D.** (2004). Translation Reinitiation at Alternative Open Reading Frames Regulates Gene Expression in an Integrated Stress Response. *J. Cell Biol.* **167**,.

**Lv, L., Sun, Y., Han, X., Xu, C., Tang, Y.-P. and Dong, Q.** (2011). Valproic Acid Improves Outcome after Rodent Spinal Cord Injury: Potential Roles of Histone Deacetylase Inhibition. *Brain Res.* **1396**, 60–68.

**Mahajan, K., Fang, B., Koomen, J. M. and Mahajan, N. P.** (2012). H2B Tyr37 Phosphorylation Suppresses Expression of Replication-dependent Core Histone Genes. *Nat. Struct. Mol. Biol.* **19**, 930–937.

**Mahat, D. B., Salamanca, H. H., Duarte, F. M., Danko, C. G. and Lis, J. T.** (2016). Mammalian Heat Shock Response and Mechanisms Underlying Its Genome-wide Transcriptional Regulation. *Mol. Cell* **62**, 63–78.

**Marinova, Z., Ren, M., Wendland, J. R., Leng, Y., Liang, M. H., Yasuda, S., Leeds, P. and Chuang, D. M.** (2009). Valproic Acid Induces Functional Heat-shock Protein 70 *via* Class I Histone Deacetylase Inhibition in Cortical Neurons: A Potential Role of Sp1 Acetylation. *J. Neurochem.* **111**, 976–987.

**Marshall, N. F. and Price, D. H.** (1995). Purification of P-TEFb, A Transcription Factor Required for the Transition Into Productive Elongation. *J. Biol. Chem.* **270**, 12335–12338.

**Mathew, R., Pal Bhadra, M. and Bhadra, U.** (2017). Insulin/insulin-like Growth Factor-1 Signalling (IIS) Based Regulation of Lifespan Across Species. *Biogerontology* **18**, 35–53.

**Mattick, J. S. and Makunin, I. V.** (2006). Non-coding RNA. *Hum. Mol. Genet.* **15**, R17–R29.

**Mayer, M. P.** (2010). Gymnastics of Molecular Chaperones. *Mol. Cell* **39**, 321–331.

**McClellan, A. J., Xia, Y., Deutschbauer, A. M., Davis, R. W., Gerstein, M. and Frydman, J.** (2007). Diverse Cellular Functions of the Hsp90 Molecular Chaperone Uncovered Using Systems Approaches. *Cell* **131**, 121–135.

**McCullough, K. D., Martindale, J. L., Klotz, L.-O., Aw, T.-Y. and Holbrook, N. J.** (2001). Gadd153 Sensitizes Cells to Endoplasmic Reticulum Stress by Down-Regulating Bcl2 and Perturbing the Cellular Redox State. *Mol. Cell. Biol.* **21**, 1249–1259.

**McMahon, M., Itoh, K., Yamamoto, M. and Hayes, J. D.** (2003). Keap1-dependent Proteasomal Degradation of Transcription Factor Nrf2 Contributes to the Negative Regulation of Antioxidant Response Element-driven Gene Expression. *J. Biol. Chem.* **278**, 21592–21600.

**McMillan, D. R., Xiao, X., Shao, L., Graves, K. and Benjamin, I. J.** (1998). Targeted Disruption of Heat Shock Transcription Factor 1 Abolishes Thermotolerance and Protection Against Heat-inducible Apoptosis. *J. Biol. Chem.* **273**, 7523–7528.

**Mendillo, M. L., Santagata, S., Koeva, M., Bell, G. W., Hu, R., Tamimi, R. M., Fraenkel, E., Ince, T. A., Whitesell, L. and Lindquist, S.** (2012). HSF1 Drives a Transcriptional Program Distinct From Heat Shock to Support Highly Malignant Human Cancers. *Cell* **150**, 549–562.

**Merath, K., Ronchetti, A. and Sidjanin, D. J.** (2013). Functional Analysis of HSF4 Mutations Found in Patients with Autosomal Recessive Congenital Cataracts. *Investig. Opthalmology Vis. Sci.* **54**, 6646–6654.

**Metchat, A., Åkerfelt, M., Bierkamp, C., Delsinne, V., Sistonen, L., Alexandre, H. and Christians, E. S.** (2009). Mammalian Heat Shock Factor 1 Is Essential for Oocyte Meiosis and Directly Regulates Hsp90alpha Expression. *J. Biol. Chem.* **284**, 9521–9528.

**Metzger, E., Yin, N., Wissmann, M., Kunowska, N., Fischer, K., Friedrichs, N., Patnaik, D., Higgins, J. M. G., Potier, N., Scheidtmann, K.-H., Buettner, R. and Schüle, R.** (2008). Phosphorylation of Histone H3 at Threonine 11 Establishes A Novel Chromatin Mark for Transcriptional Regulation. *Nat. Cell Biol.* **10**, 53–60.

**Metzger, E., Imhof, A., Patel, D., Kahl, P., Hoffmeyer, K., Friedrichs, N., Müller, J. M., Greschik, H., Kirfel, J., Ji, S., Kunowska, N., Beisenherz-Huss, C., Günther, T., Buettner, R. and Schüle, R.** (2010). Phosphorylation of Histone H3T6 by PKCβI Controls Demethylation At Histone H3K4. *Nature* **464**, 792–796.

**Mieczkowski, J., Cook, A., Bowman, S. K., Mueller, B., Alver, B. H., Kundu, S., Deaton, A. M., Urban, J. A., Larschan, E., Park, P. J., Kingston, R. E. and Tolstorukov, M. Y.** (2016). MNase Titration Reveals Differences Between Nucleosome Occupancy and Chromatin Accessibility. *Nat. Commun.* **7**, 11485.

**Milne, T. A., Briggs, S. D., Brock, H. W., Martin, M. E., Gibbs, D., Allis, C. D. and Hess, J. L.** (2002). MLL Targets SET Domain Methyltransferase Activity to Hox Gene Promoters. *Mol. Cell* **10**, 1107–1117.

**Min, J.-N., Huang, L., Zimonjic, D. B., Moskophidis, D. and Mivechi, N. F.** (2007). Selective Suppression of Lymphomas by Functional Loss of Hsf1 in A P53-deficient Mouse Model for Spontaneous Tumors. *Oncogene* **26**, 5086–5097.

**Minamiya, Y., Ono, T., Saito, H., Takahashi, N., Ito, M., Mitsui, M., Motoyama, S. and Ogawa, J.** (2011). Expression of Histone Deacetylase 1 Correlates with a Poor Prognosis in Patients with Adenocarcinoma of the Lung. *Lung Cancer* **74**, 300–304.

**Miyakawa, H., Woo, S. K., Chen, C. P., Dahl, S. C., Handler, J. S. and Kwon, H. M.** (1998). Cis- and Trans-acting Factors Regulating Transcription of the BGT1 Gene in Response to Hypertonicity. *Am. J. Physiol.* **274**, F753–F761.

**Miyakawa, H., Woo, S. K., Dahl, S. C., Handler, J. S. and Kwon, H. M.** (1999). Tonicity-responsive Enhancer Binding Protein, a Rel-like Protein that Stimulates Transcription in Response to Hypertonicity. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 2538–2542.

**Miyazaki, K., Kawamoto, T., Tanimoto, K., Nishiyama, M., Honda, H. and Kato, Y.** (2002). Identification of Functional Hypoxia Response Elements in the Promoter Region of the DEC1 and DEC2 Genes. *J. Biol. Chem.* **277**, 47014–47021.

**Mizzen, C. A., Yang, X. J., Kokubo, T., Brownell, J. E., Bannister, A. J., Owen-Hughes, T., Workman, J., Wang, L., Berger, S. L., Kouzarides, T., Nakatani, Y. and Allis, C. D.** (1996). The TAF(II)250 Subunit of TFIID Has Histone Acetyltransferase Activity. *Cell* **87**, 1261–1270.

**Morimoto, R. I.** (2011). The Heat Shock Response: Systems Biology of Proteotoxic Stress in Aging and Disease. *Cold Spring Harb. Symp. Quant. Biol.* **76**, 91–99.

**Morley, J. F. and Morimoto, R. I.** (2004). Regulation of Longevity in Caenorhabditis Elegans by Heat Shock Factor and Molecular Chaperones. *Mol. Biol. Cell* **15**, 3751–3737.

**Morton, E. A. and Lamitina, T.** (2013). Caenorhabditis Elegans HSF-1 Is an Essential Nuclear Protein that Forms Stress Granule-like Structures Following Heat Shock. *Aging Cell* **12**, 112–120.

**Mosser, D. D., Theodorakis, N. G. and Morimoto, R. I.** (1988). Coordinate Changes in Heat Shock Element-binding Activity and HSP70 Gene Transcription Rates in Human Cells. *Mol. Cell. Biol.* **8**, 4736–4744.

**Murata, M., Gong, P., Suzuki, K. and Koizumi, S.** (1999). Differential Metal Response and Regulation of Human Heavy Metal-inducible Genes. *J. Cell. Physiol.* **180**, 105–113.

**Murshid, A., Chou, S.-D., Prince, T., Zhang, Y., Bharti, A. and Calderwood, S. K.** (2010). Protein Kinase A Binds and Activates Heat Shock Factor 1. *PLoS One* **5**, e13830.

**Muse, G. W., Gilchrist, D. A., Nechaev, S., Shah, R., Parker, J. S., Grissom, S. F., Zeitlinger, J. and Adelman, K.** (2007). RNA Polymerase Is Poised for Activation Across the Genome. *Nat. Genet.* **39**, 1507–1511.

**Nakagawa, T., Zhu, H., Morishima, N., Li, E., Xu, J., Yankner, B. A. and Yuan, J.** (2000). Caspase-12 Mediates Endoplasmic-reticulum-specific Apoptosis and Cytotoxicity by Amyloid-β. *Nature* **403**, 98–103.

**Nakai, A., Tanabe, M., Kawazoe, Y., Inazawa, J., Morimoto, R. I. and Nagata, K.** (1997). HSF4, a New Member of the Human Heat Shock Factor Family Which Lacks Properties of a Transcriptional Activator. *Mol. Cell. Biol.* **17**, 469–481.

**Nanasi, P. P. and Jednakovits, A.** (2001). Multilateral *In Vivo* and *In Vitro* Protective Effects of the Novel Heat Shock Protein Coinducer, Bimoclomol: Results of Preclinical Studies. *Cardiovasc Drug Rev* **19**, 133–151.

**Nechaev, S. and Adelman, K.** (2011). Pol II Waiting in the Starting Gates: Regulating the Transition From Transcription Initiation Into Productive Elongation. *Biochim. Biophys. Acta - Gene Regul. Mech.* **1809**, 34–45.

**Nechaev, S., Fargo, D. C., dos Santos, G., Liu, L., Gao, Y. and Adelman, K.** (2010). Global Analysis of Short RNAs Reveals Widespread Promoter-proximal Stalling and Arrest of Pol II in Drosophila. *Science* **327**, 335–338.

**Neckers, L. and Workman, P.** (2012). Hsp90 Molecular Chaperone Inhibitors: Are We There Yet? *Clin. Cancer Res.* **18**, 64–76.

**Neef, D. W., Turski, M. L. and Thiele, D. J.** (2010). Modulation of Heat Shock Transcription Factor 1 as a Therapeutic Target for Small Molecule Intervention in Neurodegenerative Disease. *PLoS Biol.* **8**, e1000291.

**Neef, D. W., Jaeger, A. M. and Thiele, D. J.** (2013). Genetic Selection for Constitutively Trimerized Human HSF1 Mutants Identifies a Role for Coiled-Coil Motifs in DNA Binding. *G3* **3**, 1315–1324.

**Neef, D. W., Jaeger, A. M., Gomez-Pastor, R., Willmund, F., Frydman, J. and Thiele, D. J.** (2014). A Direct Regulatory Interaction Between Chaperonin TRiC and Stress-Responsive Transcription Factor HSF1. *Cell Rep.* **9**, 955–966.

**Neudegger, T., Verghese, J., Hayer-Hartl, M., Hartl, F. U. and Bracher, A.** (2016). Structure of Human Heat-shock Transcription Factor 1 in Complex with DNA. *Nat. Struct. Mol. Biol.* **1**, 140–146.

**Newton, E. M., Knauf, U., Green, M. and Kingston, R. E.** (1996). The Regulatory Domain of Human Heat Shock Factor 1 Is Sufficient to Sense Heat Stress. *Mol. Cell. Biol.* **16**, 839–846.

**Ng, S. S., Yue, W. W., Oppermann, U. and Klose, R. J.** (2009). Dynamic Protein Methylation in Chromatin Biology. *Cell. Mol. Life Sci.* **66**, 407–422.

**Nieto-Sotelo, J., Wiederrecht, G., Okuda, A. and Parker, C. S.** (1990). The Yeast Heat Shock Transcription Factor Contains a Transcriptional Activation Domain Whose Activity Is Repressed Under Nonshock Conditions. *Cell* **62**, 807–817.

**Nishi, H., Hashimoto, K., Panchenko, A. R., Gherardini, P. F., Tramontano, A., Helmer-Citterich, M., Via, A., Kyin, S., Muir, T. W., Fairman, R., Al., E. and Al., E.** (2011). Phosphorylation In Protein-protein Binding: Effect On Stability And Function. *Structure* **19**, 1807–1815.

**Nishino, T. G., Miyazaki, M., Hoshino, H., Miwa, Y., Horinouchi, S. and Yoshida, M.** (2008). 14-3-3 Regulates the Nuclear Import of Class IIa Histone Deacetylases. *Biochem. Biophys. Res. Commun.* **377**, 852–856.

**Nishioka, K., Chuikov, S., Sarma, K., Erdjument-Bromage, H., Allis, C. D., Tempst, P. and Reinberg, D.** (2002). Set9, A Novel Histone H3 Methyltransferase that Facilitates Transcription by Precluding Histone Tail Modifications Required for Heterochromatin Formation. *Genes Dev.* **16**, 479–489.

**Nobile, C., Nickol, J. and Martin, R. G.** (1986). Nucleosome Phasing on a DNA Fragment From the Replication

Origin of Simian Virus 40 and Rephasing Upon Cruciform Formation of the DNA. *Mol. Cell. Biol.* **6**, 2916–2922.

**Obrig, T. G., Culp, W. J., Mckeehan, W. L. and Hardesty, B.** (1971). The Mechanism by Which Cycloheximide and Related Glutarimide Antibiotics Inhibit Peptide Synthesis on Reticulocyte Ribosomes. *J. Biol. Chem.* **246**, 174–181.

**Ogryzko, V. V, Schiltz, R. L., Russanova, V., Howard, B. H. and Nakatani, Y.** (1996). The Transcriptional Coactivators P300 and CBP Are Histone Acetyltransferases. *Cell* **87**, 953–959.

**Olsen, E. A., Kim, Y. H., Kuzel, T. M., Pacheco, T. R., Foss, F. M., Parker, S., Frankel, S. R., Chen, C., Ricker, J. L., Arduino, J. M. and Duvic, M.** (2007). Phase IIB Multicenter Trial of Vorinostat in Patients with Persistent, Progressive, Or Treatment Refractory Cutaneous T-Cell Lymphoma. *J. Clin. Oncol.* **25**, 3109–3115.

**Ouararhni, K., Hadj-Slimane, R., Ait-Si-Ali, S., Robin, P., Mietton, F., Harel-Bellan, A., Dimitrov, S. and Hamiche, A.** (2006). The Histone Variant MH2A1.1 Interferes with Transcription by Down-regulating PARP-1 Enzymatic Activity. *Genes Dev.* **20**, 3324–3336.

**Ovakim, D. H. and Heikkila, J. J.** (2003). Effect of Histone Deacetylase Inhibitors on Heat Shock Protein Gene Expression During Xenopus Development. *Genesis* **36**, 88–96.

**Ozdağ, H., Teschendorff, A. E., Ahmed, A. A., Hyland, S. J., Blenkiron, C., Bobrow, L., Veerakumarasivam, A., Burtt, G., Subkhankulova, T., Arends, M. J., Collins, V. P., Bowtell, D., Kouzarides, T., Brenton, J. D. and Caldas, C.** (2006). Differential Expression of Selected Histone Modifier Genes in Human Solid Cancers. *BMC Genomics* **7**, 90.

**Pan, T., Li, X., Xie, W., Jankovic, J. and Le, W.** (2005). Valproic Acid-mediated Hsp70 Induction and Anti-apoptotic Neuroprotection in SH-SY5Y Cells. *FEBS Lett.* **579**, 6716–6720.

**Park, J. M., Werner, J., Kim, J. M., Lis, J. T. and Kim, Y. J.** (2001). Mediator, Not Holoenzyme, Is Directly Recruited to the Heat Shock Promoter by HSF Upon Heat Shock. *Mol. Cell* **8**, 9–19.

**Peart, M. J., Smyth, G. K., van Laar, R. K., Bowtell, D. D., Richon, V. M., Marks, P. A., Holloway, A. J. and Johnstone, R. W.** (2005). Identification and Functional Significance of Genes Regulated by Structurally Different Histone Deacetylase Inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 3697–3702.

**Perisic, O., Xiao, H. and Lis, J. T.** (1989). Stable Binding of Drosophila Heat Shock Factor to Head-to-head and Tail-to-tail Repeats of A Conserved 5 Bp Recognition Unit. *Cell* **59**, 797–806.

**Peteranderl, R., Rabenstein, M., Shin, Y. K., Liu, C. W., Wemmer, D. E., King, D. S. and Nelson, H. C.** (1999). Biochemical and Biophysical Characterization of the Trimerization Domain From the Heat Shock Transcription Factor. *Biochemistry* **38**, 3559–3569.

**Petesch, S. J. and Lis, J. T.** (2008). Rapid, Transcription-Independent Loss of Nucleosomes Over a Large Chromatin Domain at Hsp70 *Loci*. *Cell* **134**, 74–84.

**Petesch, S. J. and Lis, J. T.** (2012). Activator-induced Spread of Poly(ADP-Ribose) Polymerase Promotes Nucleosome Loss At Hsp70. *Mol. Cell* **45**, 64–74.

**Petrovski, G., Gurusamy, N. and Das, D. K.** (2011). Resveratrol in Cardiovascular Health and Disease. *Ann. N. Y. Acad. Sci.* **1215**, 22–33.

**Pirkkala, L., Nykanen, P. and Sistonen, L.** (2001). Roles of the Heat Shock Transcription Factors in Regulation of the Heat Shock Response and Beyond. *FASEB J.* **15**, 1118–1131.

**Pontén, F., Gry, M., Fagerberg, L., Lundberg, E., Asplund, A., Berglund, L., Oksvold, P., Björling, E., Hober, S., Kampf, C., Navani, S., Nilsson, P., Ottosson, J., Persson, A., Wernérus, H., Wester, K. and Uhlén, M.** (2009). A Global View of Protein Expression in Human Cells, Tissues, and Organs. *Mol. Syst. Biol.* **5**, 337.

**Prahlad, V., Cornelius, T. and Morimoto, R. I.** (2008). Regulation of the Cellular Heat Shock Response in Caenorhabditis Elegans by Thermosensory Neurons. *Science* **320**, 811–814.

**Rabindran, S. K., Giorgi, G., Clos, J. and Wu, C.** (1991). Molecular Cloning and Expression of a Human Heat Shock Factor, HSF1. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6906–6910.

**Racz, I., Tory, K., Gallyas, F., Berente, Z., Osz, E., Jaszlits, L., Bernath, S., Sumegi, B., Rabloczky, G. and Literati-Nagy, P.** (2002). BGP-15 - a Novel Poly(ADP-ribose) Polymerase Inhibitor - Protects Against

Nephrotoxicity of Cisplatin Without Compromising Its Antitumor Activity. *Biochem. Pharmacol.* **63**, 1099–1111.

**Rafehi, H., Balcerczyk, A., Lunke, S., Kaspi, A., Ziemann, M., KN, H., Okabe, J., Khurana, I., Ooi, J., Khan, A. W., Du, X.-J., Chang, L., Haviv, I., Keating, S. T., Karagiannis, T. C. and El-Osta, A.** (2014). Vascular Histone Deacetylation by Pharmacological HDAC Inhibition. *Genome Res.* **24**, 1271–1284.

**Rao, R. V, Castro-Obregon, S., Frankowski, H., Schuler, M., Stoka, V., del Rio, G., Bredesen, D. E. and Ellerby, H. M.** (2002). Coupling Endoplasmic Reticulum Stress to the Cell Death Program. an Apaf-1-independent Intrinsic Pathway. *J. Biol. Chem.* **277**, 21836–21842.

**Ratcliffe, P. J., Maxwell, P. H., Wiesener, M. S., Chang, G.-W., Clifford, S. C., Vaux, E. C., Cockman, M. E., Wykoff, C. C., Pugh, C. W. and Maher, E. R.** (1999). The Tumour Suppressor Protein VHL Targets Hypoxia-inducible Factors for Oxygen-dependent Proteolysis. *Nature* **399**, 271–275.

**Raychaudhuri, S., Loew, C., Körner, R., Pinkert, S., Theis, M., Hayer-Hartl, M., Buchholz, F. and Hartl, F. U.** (2014). Interplay of Acetyltransferase EP300 and the Proteasome System in Regulating Heat Shock Transcription Factor 1. *Cell* **156**, 975–985.

**Rhee, S. G., Bae, Y. S., Lee, S.-R. and Kwon, J.** (2000). Hydrogen Peroxide: a Key Messenger that Modulates Protein Phosphorylation Through Cysteine Oxidation. *Sci. Signal.* **2000**, pe1.

**Ritossa, F.** (1962). A New Puffing Pattern Induced by Temperature Shock and DNP in Drosophila. *Experientia* **18**, 571–573.

**Roche, J. and Bertrand, P.** (2016). Inside HDACs with More Selective HDAC Inhibitors. *Eur. J. Med. Chem.* **121**, 451–483.

**Rosenfeld, J. V, Maas, A. I., Bragge, P., Morganti-Kossmann, M. C., Manley, G. T. and Gruen, R. L.** (2012). Early Management of Severe Traumatic Brain Injury. *Lancet* **380**, 1088–1098.

**Ross, C. A. and Poirier, M. A.** (2004). Protein Aggregation and Neurodegenerative Disease. *Nat. Med.* **10**, S10–S17.

**Rossetto, D., Avvakumov, N. and Côté, J.** (2012). Histone Phosphorylation. *Epigenetics* **7**, 1098–1108.

**Rougvie, A. E. and Lis, J. T.** (1988). The RNA Polymerase II Molecule at the 5′ End of the Uninduced Hsp70 Gene of D. Melanogaster Is Transcriptionally Engaged. *Cell* **54**, 795–804.

**Rushmore, T. H., Morton, M. R. and Pickett, C. B.** (1991). The Antioxidant Responsive Element: Activation by Oxidative Stress and Identification of the DNA Consensus Sequence Required for Functional Activity. *J. Biol. Chem.* **266**, 11632–11639.

**Sadowski, I., Ma, J., Triezenberg, S. and Ptashne, M.** (1988). GAL4-VP16 Is an Unusually Potent Transcriptional Activator. *Nature* **335**, 563–564.

**Salah, H., Li, M., Cacciani, N., Gastaldello, S., Ogilvie, H., Akkad, H., Namuduri, A. V, Morbidoni, V., Artemenko, K. A., Balogh, G., Martinez-Redondo, V., Jannig, P., Hedstrom, Y., Dworkin, B., Bergquist, J., Ruas, J., Vigh, L., Salviati, L. and Larsson, L.** (2016). The Chaperone Co-inducer BGP-15 Alleviates Ventilation-induced Diaphragm Dysfunction. *Sci. Transl. Med.* **8**, 350ra103.

**Salceda, S. and Caro, J.** (1997). Hypoxia-inducible Factor 1 (HIF-1 ) Protein Is Rapidly Degraded by the Ubiquitin-Proteasome System Under Normoxic Conditions: Its Stabilization by Hypoxia Dpends on Redox-induced Changes. *J. Biol. Chem.* **272**, 22642–22647.

**Sandqvist, A. and Sistonen, L.** (2004). Nuclear Stress Granules: the Awakening of a Sleeping Beauty? *J. Cell Biol.* **164**, 15–17.

**Santagata, S., Hu, R., Lin, N. U., Mendillo, M. L., Collins, L. C., Hankinson, S. E., Schnitt, S. J., Whitesell, L., Tamimi, R. M., Lindquist, S. and Ince, T. A.** (2011). High Levels of Nuclear Heat-shock Factor 1 (HSF1) Are Associated with Poor Prognosis in Breast Cancer. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 18378–18383.

**Santagata, S., Mendillo, M. L., Tang, Y. -c., Subramanian, A., Perley, C. C., Roche, S. P., Wong, B., Narayan, R., Kwon, H., Koeva, M., Amon, A., Golub, T. R., Porco, J. A., Whitesell, L. and Lindquist, S.** (2013). Tight Coordination of Protein Translation and HSF1 Activation Supports the Anabolic Malignant State. *Science* **341**, 1238303.

**Santos, S. D. and Saraiva, M. J.** (2004). Enlarged Ventricles, Astrogliosis and Neurodegeneration in Heat Shock

Factor 1 Null Mouse Brain. *Neuroscience* **126**, 657–663.

**Sapra, G., Tham, Y. K., Cemerlang, N., Matsumoto, A., Kiriazis, H., Bernardo, B. C., Henstridge, D. C., Ooi, J. Y. Y., Pretorius, L., Boey, E. J. H., Lim, L., Sadoshima, J., Meikle, P. J., Mellet, N. a., Woodcock, E. a., Marasco, S., Ueyama, T., Du, X.-J., Febbraio, M. A. and McMullen, J.R.** (2014). The Small-molecule BGP-15 Protects Against Heart Failure and Atrial Fibrillation in Mice. *Nat. Commun.* **5**, 5705.

**Sarge, K. D., Murphy, S. P. and Morimoto, R. I.** (1993). 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**, 1392–1407.

**Scharf, K.-D., Berberich, T., Ebersberger, I. and Nover, L.** (2012). The Plant Heat Stress Transcription Factor (Hsf) Family: Structure, Function and Evolution. *Biochim. Biophys. Acta - Gene Regul. Mech.* **1819**, 104–119.

**Scherz-Shouval, R., Santagata, S., Mendillo, M. L., Sholl, L. M., Ben-Aharon, I., Beck, A. H., Dias-Santagata, D., Koeva, M., Stemmer, S. M., Whitesell, L. and Lindquist, S.** (2014). The Reprogramming of Tumor Stroma by HSF1 Is a Potent Enabler of Malignancy. *Cell* **158**, 564–578.

**Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P. and Cardona, A.** (2012). Fiji: An Open-source Platform for Biological-image Analysis. *Nat. Methods* **9**, 676–682.

**Schlick, T., Hayes, J. and Grigoryev, S.** (2012). Toward Convergence of Experimental Studies and Theoretical Modeling of the Chromatin Fiber. *J. Biol. Chem.* **287**, 5183–5191.

**Schröder, M. and Kaufman, R. J.** (2005). The Mammalian Unfolded Protein Response. *Annu. Rev. Biochem.* **74**, 739–789.

**Schuettengruber, B., Simboeck, E., Khier, H. and Seiser, C.** (2003). Autoregulation of Mouse Histone Deacetylase 1 Expression. *Mol. Cell. Biol.* **23**, 6993–7004.

**Schuetz, T. J., Gallo, G. J., Sheldon, L., Tempst, P. and Kingston, R. E.** (1991). Isolation of a CDNA for HSF2: Evidence for Two Heat Shock Factor Genes in Humans. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6911–6915.

**Sena, L. A. and Chandel, N. S.** (2012). Physiological Roles of Mitochondrial Reactive Oxygen Species. *Mol. Cell* **48**, 158–167.

**Shah, S. P., Lonial, S. and Boise, L. H.** (2015). Inhibition of Heat Shock Factor 1 (HSF1) Is More Effective at Sensitizing Myeloma Cells to Bortezomib than Inhibition of Individual HSF1 Targets. *Blood* **120**, 2953.

**Shah, S. P., Nooka, A. K., Jaye, D. L., Bahlis, N. J., Lonial, S. and Boise, L. H.** (2016). Bortezomib-induced Heat Shock Response Protects Multiple Myeloma Cells and Is Activated by Heat Shock Factor 1 Serine 326 Phosphorylation. *Oncotarget* **5**, 59727–59741.

**Shen, J., Chen, X., Hendershot, L. and Prywes, R.** (2002). ER Stress Regulation of ATF6 Localization by Dissociation of BiP/GRP78 Binding and Unmasking of Golgi Localization Signals. *Dev. Cell* **3**, 99–111.

**Shi, Y., Kroeger, P. E. and Morimoto, R. I.** (1995). The Carboxyl-terminal Transactivation Domain of Heat Shock Factor 1 Is Negatively Regulated and Stress Responsive. *Mol. Cell. Biol.* **15**, 4309–4318.

**Shi, Y., Mosser, D. D. and Morimoto, R. I.** (1998). Molecular Chaperones as HSF1-specific Transcriptional Repressors. *Genes Dev.* **12**, 654–666.

**Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J. R., Cole, P. A., Casero, R. A. and Shi, Y.** (2004). Histone Demethylation Mediated by the Nuclear Amine Oxidase Homolog LSD1. *Cell* **119**, 941–953.

**Shi, X., Hong, T., Walter, K. L., Ewalt, M., Michishita, E., Hung, T., Carney, D., Peña, P., Lan, F., Kaadige, M. R., Lacoste, N., Cayrou, C., Davrazou, F., Saha, A., Cairns, B. R., Ayer, D. E., Kutateladze, T. G., Shi, Y., Côté, J., Chua, K.F. and Gozani, O.** (2006). ING2 PHD Domain Links Histone H3 Lysine 4 Methylation to Active Gene Repression. *Nature* **442**, 96–99.

**Shi, Y., Shi, X., Jin, Y., Miao, A., Bu, L., He, J., Jiang, H., Lu, Y., Kong, X. and Hu, L.** (2008). Mutation Screening of HSF4 in 150 Age-related Cataract Patients. *Mol. Vis.* **14**, 1850–1855.

**Shi, X., Cui, B., Wang, Z., Weng, L., Xu, Z., Ma, J., Xu, G., Kong, X. and Hu, L.** (2009). Removal of Hsf4 Leads to Cataract Development in Mice Through Down-regulation of ΓS-crystallin and Bfsp Expression. *BMC Mol. Biol.* **10**, 10.

**Shiio, Y. and Eisenman, R. N.** (2003). Histone Sumoylation Is Associated with Transcriptional Repression. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 13225–13230.

**Shinka, T., Sato, Y., Chen, G., Naroda, T., Kinoshita, K., Unemi, Y., Tsuji, K., Toida, K., Iwamoto, T. and Nakahori, Y.** (2004). Molecular Characterization of Heat Shock-like Factor Encoded on the Human Y Chromosome, and Implications for Male Infertility. *Biol. Reprod.* **71**, 297–306.

**Shinkawa, T., Tan, K., Fujimoto, M., Hayashida, N., Yamamoto, K., Takaki, E., Takii, R., Prakasam, R., Inouye, S., Mezger, V. and Nakai, A.** (2011). Heat Shock Factor 2 Is Required for Maintaining Proteostasis Against Febrile-range Thermal Stress and Polyglutamine Aggregation. *Mol. Biol. Cell* **22**, 3571–3583.

**Shivaswamy, S. and Iyer, V. R.** (2008). Stress-dependent Dynamics of Global Chromatin Remodeling in Yeast: Dual Role for SWI/SNF in the Heat Shock Stress Response. *Mol. Cell. Biol.* **28**, 2221–2234.

**Sinn, D.-I., Kim, S.-J., Chu, K., Jung, K.-H., Lee, S.-T., Song, E.-C., Kim, J.-M., Park, D.-K., Kun Lee, S., Kim, M. and Roh, J.-K.** (2007). Valproic Acid-mediated Neuroprotection in Intracerebral Hemorrhage *Via* Histone Deacetylase Inhibition and Transcriptional Activation. *Neurobiol. Dis.* **26**, 464–472.

**Smolle, M. and Workman, J. L.** (2013). Transcription-associated Histone Modifications and Cryptic Transcription. *Biochim. Biophys. Acta - Gene Regul. Mech.* **1829**, 84–97.

**Solimini, N. L., Luo, J. and Elledge, S. J.** (2007). Non-Oncogene Addiction and the Stress Phenotype of Cancer Cells. *Cell* **130**, 986–988.

**Solís, E. J., Pandey, J. P., Zheng, X., Jin, D. X., Gupta, P. B., Airoldi, E. M., Pincus, D. and Denic, V.** (2016). Defining the Essential Function of Yeast Hsf1 Reveals a Compact Transcriptional Program for Maintaining Eukaryotic Proteostasis. *Mol. Cell* **63**, 60–71.

**Soncin, F., Zhang, X., Chu, B., Wang, X., Asea, A., Ann Stevenson, M., Sacks, D. B. and Calderwood, S. K.** (2003). Transcriptional Activity and DNA Binding of Heat Shock Factor-1 Involve Phosphorylation on Threonine 142 by CK2. *Biochem. Biophys. Res. Commun.* **303**, 700–706.

**Sorger, P. K. and Pelham, H. R. B.** (1988). Yeast Heat Shock Factor Is an Essential DNA-binding Protein that Exhibits Temperature-dependent Phosphorylation. *Cell* **54**, 855–864.

**Stanhill, A., Levin, V., Hendel, A., Shachar, I., Kazanov, D., Arber, N., Kaminski, N. and Engelberg, D.** (2006). Ha-Rasval12 Induces HSP70b Transcription *Via* the HSE/HSF1 System, but HSP70b Expression Is Suppressed in Ha-Rasval12-transformed Cells. *Oncogene* **25**, 1485–1495.

**Stulemeijer, I. J. E., De Vos, D., van Harten, K., Joshi, O. K., Blomberg, O., van Welsem, T., Terweij, M., Vlaming, H., de Graaf, E. L., Altelaar, A. F. M., Bakker, B. M. and van Leeuwen, F.** (2015). Dot1 Histone Methyltransferases Share a Distributive Mechanism but Have Highly Diverged Catalytic Properties. *Sci. Rep.* **5**, 9824.

**Su, K.-H., Cao, J., Tang, Z., Dai, S., He, Y., Sampson, S. B., Benjamin, I. J. and Dai, C.** (2016). HSF1 Critically Attunes Proteotoxic Stress Sensing by mTORC1 to Combat Stress and Promote Growth. *Nat. Cell Biol.* **18**, 527–539.

**Sullivan, E. K., Weirich, C. S., Guyon, J. R., Sif, S. and Kingston, R. E.** (2001). Transcriptional Activation Domains of Human Heat Shock Factor 1 Recruit Human SWI/SNF. *Mol. Cell. Biol.* **21**, 5826–5837.

**Sumegi, K., Fekete, K., Antus, C., Debreceni, B., Hocsak, E., Gallyas, F., Sumegi, B. and Szabo, A.** (2017). BGP-15 Protects Against Oxidative Stress- or Lipopolysaccharide-Induced Mitochondrial Destabilization and Reduces Mitochondrial Production of Reactive Oxygen Species. *PLoS One* **12**, e0169372.

**Tachibana, M., Sugimoto, K., Fukushima, T. and Shinkai, Y.** (2001). SET Domain-containing Protein, G9a, Is a Novel Lysine-preferring Mammalian Histone Methyltransferase with Hyperactivity and Specific Selectivity to Lysines 9 and 27 of Histone H3. *J. Biol. Chem.* **276**, 25309–25317.

**Taipale, M., Krykbaeva, I., Koeva, M., Kayatekin, C., Westover, K. D., Karras, G. I. and Lindquist, S.** (2012). Quantitative Analysis of HSP90-client Interactions Reveals Principles of Substrate Recognition. *Cell* **150**, 987–1001.

**Taipale, M., Tucker, G., Peng, J., Krykbaeva, I., Lin, Z.-Y., Larsen, B., Choi, H., Berger, B., Gingras, A.-C. and Lindquist, S.** (2014). A Quantitative Chaperone Interaction Network Reveals the Architecture of Cellular Protein Homeostasis Pathways. Cell 158, 434–448.

**Takahashi, H., Parmely, T. J., Sato, S., Tomomori-Sato, C., Banks, C. A. S., Kong, S. E., Szutorisz, H., Swanson, S. K., Martin-Brown, S., Washburn, M. P., Florens, L., Seidel, C. W., Lin, C., Smith, E. R., Shilatifard, A., Conaway, R. C. and Conaway, J. W.** (2011). Human Mediator Subunit MED26 Functions as a Docking Site for Transcription Elongation Factors. *Cell* **146**, 92–104.

**Takii, R., Inouye, S., Fujimoto, M., Nakamura, T., Shinkawa, T., Prakasam, R., Tan, K., Hayashida, N., Ichikawa, H., Hai, T. and Nakai, A.** (2010). Heat Shock Transcription Factor 1 Inhibits Expression of IL-6 Through Activating Transcription Factor 3. *J. Immunol.* **184**, 1041–1048.

**Takii, R., Fujimoto, M., Tan, K., Takaki, E., Hayashida, N., Nakato, R., Shirahige, K. and Nakai, A.** (2015). ATF1 Modulates the Heat Shock Response by Regulating the Stress-Inducible Heat Shock Factor 1 Transcription Complex. *Mol. Cell. Biol.* **35**, 11–25.

**Tang, Y., Zhao, W., Chen, Y., Zhao, Y. and Gu, W.** (2008). Acetylation Is Indispensable for P53 Activation. *Cell* **133**, 612–626.

**Tang, Z., Dai, S., He, Y., Doty, R. A., Shultz, L. D., Sampson, S. B. and Dai, C.** (2015). MEK Guards Proteome Stability and Inhibits Tumor-suppressive Amyloidogenesis Via HSF1. *Cell* **160**, 729–744.

**Tatum, M. C., Ooi, F. K., Chikka, M. R., Chauve, L., Martinez-Velazquez, L. A., Steinbusch, H. W. M., Morimoto, R. I. and Prahlad, V.** (2015). Neuronal Serotonin Release Triggers the Heat Shock Response in C. elegans in the Absence of Temperature Increase. *Curr. Biol.* **25**, 163–174.

**Taunton, J., Hassig, C. A. and Schreiber, S. L.** (1996). A Mammalian Histone Deacetylase Related to the Yeast Transcriptional Regulator Rpd3p. *Science* **272**, 408–411.

**Tessarz, P. and Kouzarides, T.** (2014). Histone Core Modifications Regulating Nucleosome Structure and Dynamics. *Nat. Rev. Mol. Cell Biol.* **15**, 703–708.

**Thomas, M. C. and Chiang, C.-M.** (2006). The General Transcription Machinery and General Cofactors. *Crit. Rev. Biochem. Mol. Biol.* **41**, 105–178.

**Thomas, I. and Gregg, B.** (2017). Metformin; A Review of Its History and Future: From Lilac to Longevity. *Pediatr. Diabetes* **18**, 10–16.

**Tissières, A., Mitchell, H. K. and Tracy, U. M.** (1974). Protein Synthesis in Salivary Glands of Drosophila Melanogaster: Relation to Chromosome Puffs. *J. Mol. Biol.* **84**, 389–398.

**Trama, J., Lu, Q., Hawley, R. G. and Ho, S. N.** (2000). The NFAT-related Protein NFATL1 (TonEBP/NFAT5) Is Induced Upon T Cell Activation in a Calcineurin-dependent Manner. *J. Immunol.* **165**, 4884–4894.

**Tsukada, Y., Fang, J., Erdjument-Bromage, H., Warren, M. E., Borchers, C. H., Tempst, P. and Zhang, Y.** (2005). Histone Demethylation by a Family of JmjC Domain-containing Proteins. *Nature* **439**, 811–816.

**Uchida, S., Nakanishi, T., Kwon, H. M., Preston, A. S. and Handler, J. S.** (1991). Taurine Behaves as an Osmolyte in Madin-Darby Canine Kidney Cells. Protection by Polarized, Regulated Transport of Taurine. *J. Clin. Invest.* **88**, 656–662.

**Uchida, S., Hara, K., Kobayashi, A., Fujimoto, M., Otsuki, K., Yamagata, H., Hobara, T., Abe, N., Higuchi, F., Shibata, T., Hasegawa, S., Kida, S., Nakai, A. and Watanabe, Y.** (2011). Impaired Hippocampal Spinogenesis and Neurogenesis and Altered Affective Behavior in Mice Lacking Heat Shock Factor 1. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 1681–1686.

**Urano, F., Wang, X., Bertolotti, A., Zhang, Y., Chung, P., Harding, H. P. and Ron, D.** (2000). Coupling of Stress in the ER to Activation of JNK Protein Kinases by Transmembrane Protein Kinase IRE1. *Science* **287**, 664–666.

**van der Giezen, M.** (2011). Mitochondria and the Rise of Eukaryotes. *Bioscience* **61**, 594–601.

**Verma, P., Pfister, J. A., Mallick, S. and D'Mello, S. R.** (2014). HSF1 Protects Neurons Through a Novel Trimerization- and HSP-Independent Mechanism. *J. Neurosci.* **34**, 1599–1612.

**Vígh, L., Literáti, P. N., Horváth, I., Török, Z., Balogh, G., Glatz, A., Kovács, E., Boros, I., Ferdinándy, P., Parkas, B., Jaszlits, L., Jednákovits, A., Korányi, L. and Maresca, B.** (1997). Bimoclomol: A Nontoxic, Hydroxylamine Derivative with Stress Protein-inducing Activity and Cytoprotective Effects. *Nat. Med.* **3**, 1150–1154.

**Vihervaara, A., Sergelius, C., Vasara, J., Blom, M. A. H., Elsing, A. N., Roos-Mattjus, P. and Sistonen, L.** (2013).

Transcriptional Response to Stress in the Dynamic Chromatin Environment of Cycling and Mitotic Cells. *Proc. Natl. Acad. Sci. U.S.A.* **110**, E3388–E3397.

**Voellmy, R.** (2004). On Mechanisms that Control Heat Shock Transcription Factor Activity in Metazoan Cells. *Cell Stress Chaperones* **9**, 122–133.

**Vuister, G. W., Kim, S.-J., Orosz, A., Marquardt, J., Wu, C. and Bax, A.** (1994). Solution Structure of the DNA-binding Domain of Drosophila Heat Shock Transcription Factor. *Nat. Struct. Biol.* **1**, 605–614.

**Vujanac, M., Fenaroli, A. and Zimarino, V.** (2005). Constitutive Nuclear Import and Stress-Regulated Nucleocytoplasmic Shuttling of Mammalian Heat-Shock Factor 1. *Traffic* **6**, 214–229.

**Wada, T., Takagi, T., Yamaguchi, Y., Watanabe, D. and Handa, H.** (1998). Evidence that P-TEFb Alleviates the Negative Effect of DSIF on RNA Polymerase II-dependent Transcription in Vitro. *EMBO J.* **17**, 7395–7403.

**Wang, S., Diller, K. R. and Aggarwal, S. J.** (2003a). Kinetics Study of Endogenous Heat Shock Protein 70 Expression. *J. Biomech. Eng.* **125**, 794–797.

**Wang, G., Zhang, J., Moskophidis, D. and Mivechi, N. F.** (2003b). Targeted Disruption of the Heat Shock Transcription Factor (Hsf)-2 Gene Results in Increased Embryonic Lethality, Neuronal Defects, and Reduced Spermatogenesis. *Genesis* **36**, 48–61.

**Wang, X., Grammatikakis, N., Siganou, A. and Calderwood, S. K.** (2003c). Regulation of Molecular Chaperone Gene Transcription Involves the Serine Phosphorylation, 14-3-3 Binding, and Cytoplasmic Sequestration of Heat Shock Factor 1. *Mol. Cell. Biol.* **23**, 6013–6026.

**Wang, X., Khaleque, M. A., Zhao, M. J., Zhong, R., Gaestel, M. and Calderwood, S. K.** (2006). Phosphorylation of HSF1 by MAPK-Activated Protein Kinase 2 on Serine 121, Inhibits Transcriptional Activity and Promotes HSP90 Binding. *J. Biol. Chem.* **281**, 782–791.

**Wang, H., Wang, L., Erdjument-Bromage, H., Vidal, M., Tempst, P., Jones, R. S. and Zhang, Y.** (2004). Role of Histone H2A Ubiquitination in Polycomb Silencing. *Nature* **431**, 873–878.

**Weber, J. A., Taxman, D. J., Lu, Q. and Gilmour, D. S.** (1997). Molecular Architecture of the Hsp70 Promoter After Deletion of the TATA Box or the Upstream Regulation Region. *Mol. Cell. Biol.* **17**, 3799–3808.

**Weichert, W., Röske, A., Gekeler, V., Beckers, T., Stephan, C., Jung, K., Fritzsche, F. R., Niesporek, S., Denkert, C., Dietel, M. and Kristiansen, G.** (2008). Histone Deacetylases 1, 2 and 3 Are Highly Expressed in Prostate Cancer and HDAC2 Expression Is Associated with Shorter PSA Relapse Time After Radical Prostatectomy. *Br. J. Cancer* **98**, 604–610.

**West, A. C. and Johnstone, R. W.** (2014). New and Emerging HDAC Inhibitors for Cancer Treatment. *J. Clin. Invest.* **124**, 30–39.

**West, J. D., Wang, Y. and Morano, K. A.** (2012). Small Molecule Activators of the Heat Shock Response: Chemical Properties, Molecular Targets, and Therapeutic Promise. *Chem. Res. Toxicol.* **25**, 2036–2053.

**Westerheide, S. D., Anckar, J., Stevens, S. M., Sistonen, L. and Morimoto, R. I.** (2009). Stress-Inducible Regulation of Heat Shock Factor 1 by the Deacetylase SIRT1. *Science* **323**, 1063–1066.

**Westwood, J. T. and Wu, C.** (1993). Activation of Drosophila Heat Shock Factor: Conformational Change Associated with A Monomer-to-trimer Transition. *Mol. Cell. Biol.* **13**, 3481–3486.

**Whitesell, L. and Lindquist, S.** (2009). Inhibiting the Transcription Factor HSF1 as an Anticancer Strategy. *Expert Opin. Ther. Targets* **13**, 469–478.

**Whittaker, S. J., Demierre, M.-F., Kim, E. J., Rook, A. H., Lerner, A., Duvic, M., Scarisbrick, J., Reddy, S., Robak, T., Becker, J. C., Samtsov, A., McCulloch, W. and Kim, Y. H.** (2010). Final Results From A Multicenter, International, Pivotal Study of Romidepsin in Refractory Cutaneous T-Cell Lymphoma. *J. Clin. Oncol.* **28**, 4485–4491.

**Wiederrecht, G., Shuey, D. J., Kibbe, W. A. and Parker, C. S.** (1987). The Saccharomyces and Drosophila Heat Shock Transcription Factors Are Identical in Size and DNA Binding Properties. *Cell* **48**, 507–515.

**Wilmink, G. J., Roth, C. L., Ibey, B. L., Ketchum, N., Bernhard, J., Cerna, C. Z. and Roach, W. P.** (2010). Identification of MicroRNas associated with Hyperthermia-induced Cellular Stress Response. *Cell Stress Chaperones* **15**, 1027–38.

**Workman, J. L.** (2006). Nucleosome Displacement in Transcription. *Genes Dev.* **20**, 2009–2017.

**Woychik, N. A. and Hampsey, M.** (2002). The RNA Polymerase II Machinery: Structure Illuminates Function. *Cell* **108**, 453–463.

**Wu, C.** (1980). The 5′ Ends of Drosophila Heat Shock Genes in Chromatin Are Hypersensitive to DNase I. *Nature* **286**, 854–860.

**Wu, C., Wilson, S., Walker, B., Dawid, I., Paisley, T., Zimarino, V. and Ueda, H.** (1987). Purification and Properties of Drosophila Heat Shock Activator Protein. *Science* **238**, 1247–1253.

**Xia, W. and Voellmy, R.** (1997). Hyperphosphorylation of Heat Shock Transcription Factor 1 Is Correlated with Transcriptional Competence and Slow Dissociation of Active Factor Trimers. *J. Biol. Chem.* **272**, 4094–4102.

**Xiao, X., Zuo, X., Davis, A. A., McMillan, D. R., Curry, B. B., Richardson, J. A. and Benjamin, I. J.** (1999). HSF1 Is Required for Extra-embryonic Development, Postnatal Growth and Protection During Inflammatory Responses in Mice. *EMBO J.* **18**, 5943–5952.

**Xu, Y., Huang, D., Chiu, J. and Lau, A. T. Y.** (2012). Post-Translational Modification of Human Heat Shock Factors and Their Functions: A Recent Update by Proteomic Approach. *J. Proteome Res.* **11**, 2625–2634.

**Xuan, A., Long, D., Li, J., Ji, W., Hong, L., Zhang, M. and Zhang, W.** (2012). Neuroprotective Effects of Valproic Acid Following Transient Global Ischemia in Rats. *Life Sci.* **90**, 463–468.

**Yang, X.-J., Ogryzko, V. V., Nishikawa, J., Howard, B. H. and Nakatani, Y.** (1996). A P300/CBP-associated Factor that Competes with the Adenoviral Oncoprotein E1A. *Nature* **382**, 319–324.

**Yang, W., Cui, M., Lee, J., Gong, W., Wang, S., Fu, J., Wu, G. and Yan, K.** (2016). Heat Shock Protein Inhibitor, Quercetin, as a Novel Adjuvant Agent to Improve Radiofrequency Ablation-induced Tumor Destruction and Its Molecular Mechanism. *Chin. J. Cancer Res.* **28**, 19–28.

**Yenari, M. A., Liu, J., Zheng, Z., Vexler, Z. S., Lee, J. E. and Giffard, R. G.** (2005). Antiapoptotic and Anti-inflammatory Mechanisms of Heat-Shock Protein Protection. *Ann. N. Y. Acad. Sci.* **1053**, 74–83.

**Yoshida, H., Haze, K., Yanagi, H., Yura, T. and Mori, K.** (1998). Identification of the Cis-acting Endoplasmic Reticulum Stress Response Element Responsible for Transcriptional Induction of Mammalian Glucose-regulated Proteins. Involvement of Basic Leucine Zipper Transcription Factors. *J. Biol. Chem.* **273**, 33741–33749.

**Yoshida, H., Matsui, T., Yamamoto, A., Okada, T. and Mori, K.** (2001). XBP1 mRNA Is Induced by ATF6 and Spliced by IRE1 in Response to ER Stress to Produce A Highly Active Transcription Factor. *Cell* **107**, 881–891.

**Yoshidas, M., Yoshida, M., Kijima, M., Akita, M. and Beppu, T.** (1990). Potent and Specific Inhibition of Mammalian Histone Deacetylase both *In Vivo* and *In Vitro* by Trichostatin A. *J. Biol. Chem.* **265**, 17174–17179.

**Yoshima, T., Yura, T. and Yanagi, H.** (1998). Function of the C-terminal Transactivation Domain of Human Heat Shock Factor 2 Is Modulated by the Adjacent Negative Regulatory Segment. *Nucleic Acids Res.* **26**, 2580–2585.

**Yutani, K., Ogasahara, K., Tsujita, T. and Sugino, Y.** (1987). Dependence of Conformational Stability on Hydrophobicity of the Amino Acid Residue in a Series of Variant Proteins Substituted at a Unique Position of Tryptophan Synthase Alpha Subunit. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4441–4444.

**Zanini, C., Giribaldi, G., Mandili, G., Carta, F., Crescenzio, N., Bisaro, B., Doria, A., Foglia, L., Di Montezemolo, L. C., Timeus, F. and Turrini, F.** (2007). Inhibition of Heat Shock Proteins (HSP) Expression by Quercetin and Differential Doxorubicin Sensitization in Neuroblastoma and Ewing's Sarcoma Cell Lines. *J. Neurochem.* **103**, 1344–1354.

**Zegerman, P., Canas, B., Pappin, D. and Kouzarides, T.** (2002). Histone H3 Lysine 4 Methylation Disrupts Binding of Nucleosome Remodeling and Deacetylase (NuRD) Repressor Complex. *J. Biol. Chem.* **277**, 11621–11624.

**Zelin, E. and Freeman, B. C.** (2015). Lysine Deacetylases Regulate the Heat Shock Response Including the Age-associated Impairment of HSF1. *J. Mol. Biol.* **427**, 1644–1654.

**Zhang, Z. and Cohen, D. M.** (1997). Hypotonicity Increases Transcription, Expression, and Action of Egr-1 in

Murine Renal Medullary MIMCD3 Cells. *Am. J. Physiol.* **273**, 837–842.

**Zhang, D. D. and Hannink, M.** (2003). Distinct Cysteine Residues in Keap1 Are Required for Keap1-Dependent Ubiquitination of Nrf2 and for Stabilization of Nrf2 by Chemopreventive Agents and Oxidative Stress. *Mol. Cell. Biol.* **23**, 8137–8151.

**Zhang, L., Jin, M., Margariti, A., Wang, G., Luo, Z., Zampetaki, A., Zeng, L., Ye, S., Zhu, J. and Xiao, Q.** (2010). Sp1-dependent Activation of HDAC7 Is Required for Platelet-derived Growth Factor-BB-induced Smooth Muscle Cell Differentiation From Stem Cells. *J. Biol. Chem.* **285**, 38463–38472.

**Zhao, Q., Wang, J., Levichkin, I. V., Stasinopoulos, S., Ryan, M. T. and Hoogenraad, N. J.** (2002). A Mitochondrial Specific Stress Response in Mammalian Cells. *EMBO J.* **21**, 4411–4419.

**Zhao, Y. M., Chen, X., Sun, H., Yuan, Z. G., Ren, G. L., Li, X. X., Lu, J. and Huang, B. Q.** (2006). Effects of Histone Deacetylase Inhibitors on Transcriptional Regulation of the Hsp70 Gene in Drosophila. *Cell Res.* **16**, 566–576.

**Zhao, Y. H., Zhou, M., Liu, H., Ding, Y., Khong, H. T., Yu, D., Fodstad, O. and Tan, M.** (2009). Upregulation of Lactate Dehydrogenase A by ErbB2 Through Heat Shock Factor 1 Promotes Breast Cancer Cell Glycolysis and Growth. *Oncogene* **28**, 3689–3701.

**Zheng, X., Krakowiak, J., Patel, N., Beyzavi, A., Ezike, J., Khalil, A. S. and Pincus, D.** (2016). Dynamic Control of Hsf1 During Heat Shock by a Chaperone Switch and Phosphorylation. *Elife* **5**, e18638.

**Zhong, M., Orosz, A. and Wu, C.** (1998). Direct Sensing of Heat and Oxidation by Drosophila Heat Shock Transcription Factor. *Mol. Cell* **2**, 101–108.

**Zhong, S., Goto, H., Inagaki, M. and Dong, Z.** (2003). Phosphorylation At Serine 28 and Acetylation at Lysine 9 of Histone H3 Induced by Trichostatin A. *Oncogene* **22**, 5291–5297.

**Zhu, Z. and Mivechi, N. F.** (1999). Regulatory Domain of Human Heat Shock Transcription Factor-2 Is Not Regulated by Hemin or Heat Shock. *J. Cell. Biochem.* **73**, 56–69.

**Ziemka-Nalecz, M. and Zalewska, T.** (2014). Neuroprotective Effects of Histone Deacetylase Inhibitors in Brain Ischemia. *Acta Neurobiol Exp* **74**, 383–395.

**Zou, J., Guo, Y., Guettouche, T., Smith, D. F. and Voellmy, R.** (1998). Repression of Heat Shock Transcription Factor HSF1 Activation by HSP90 (HSP90 Complex) that Forms a Stress-sensitive Complex with HSF1. *Cell* **94**, 471–480.

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