Chapter 3
Regulation of Heat Shock Genes Expression

Cellular stresses modulate intracellular signaling pathways that control almost all aspects of cell physiology and metabolism. Heat shock genes system activation in response to various forms of stress is extremely rapid and, hence, represents an excellent model for investigation of gene regulation at all levels. In *Drosophila* it is possible to detect local chromatin decondensation leading to puffs formation in salivary glands polytene chromosomes within the first 1–2 min after HS challenge. Fast activation of *Hsp* genes is apparently necessary for cell survival under stress conditions and for cross-protection against unrelated stresses. Activation of HS system is rapidly initiated at all stages of genetic information realization including transcription, export of mRNA and translation.

In *E. coli* and other prokaryotes regulation of genes activity depends on RNA polymerase σ-factors that are able to recognize specific nucleotide sequences and direct RNA-polymerase to specific promoters. In *E. coli* under normal temperature conditions low level transcription of HS genes *groES* and *groEL* is maintained by major vegetative factor σ70. After temperature elevation complex of RNA polymerase with σ70 becomes destabilized and is substituted by a complex with recruited σ32. The σ32 is a product of *rpoH* gene, and it recognizes a specific motif in the promoters of heat-regulated genes. The level of *Hsp* genes expression in *E. coli* positively correlates with intercellular concentration of σ32. In the absence of stress σ32 is not able to induce significant transcription primarily because its half-life is less than 30 s due to effective proteolysis of σ32 with the involvement of proteases HflB and ClpP (Segal and Ron 1998). Under stressful conditions a pronounced increase in the quantity of misfolded and damaged proteins occurs. Such abnormal proteins produced after stress serve as target for the different intracellular proteases. As a result HflB and ClpP proteases switch to other substrates and half-life of σ32 is significantly increased. Besides, HS leads to significant increase in σ32 translation efficacy. Elevated temperature induces melting of hairpin structure located in σ32-factor 5′-mRNA and, thus, intensifies the translation process (Morita et al. 1999). Therefore, the regulation of σ32 concentration in the prokaryotic cells is realized at transcriptional and post-transcriptional levels.

M.B. Evgen'ev et al., *Heat Shock Proteins and Whole Body Adaptation to Extreme Environments*, DOI 10.1007/978-94-017-9235-6_3

In *Hsp* genes of all eukaryotes at the 5′-regulatory region upstream of TATA box there are specific motifs, the so call "heat shock elements" (HSEs). The optimal context of such sequences represents 5′-nnGAAnnTTCnnGAAnn-3′, where n – any nucleotide (Amin et al. 1988). The major functional unit in this sequence is either GAA or TTC motif. Besides this typical HSE there are so-called "gap-type HSEs" consisting of two inverted units separated from a third unit by a 5-bps gap (nTTCnnGAAn(5 bps)nGAAn), and "step-type HSEs" consisting of direct repeats of nGAAn or nTTCn motifs separated by 5 bps (nGAAn(5 bps)nGAAn(5 bps) nGAAn) (Hashikawa et al. 2006; Yamamoto et al. 2005). In addition to HSEs, *Hsp* genes frequently contain TATA-box and certain other species-specific elements. Besides HSEs, in most cases *HSP70* genes contain Sp1 binding sites and CCAAT motif in the human *HSP70* genes or GAGA motifs in *Drosophila* species. While one copy of the complete HSE sequence (see above) is sufficient for heat shock induction most of the investigated promoters of *HSP70* genes contain several HSEs located at different distances from the transcription start. As an example, *Hsp70* genes of *D. melanogaster* contain four HSEs (Amin et al. 1987; Tian et al. 2010). Various lines of evidence demonstrated that the increase in HSEs copy number in the promoters of *Hsp* genes significantly enhance their transcription (Amin et al. 1988, 1994; Lerman and Feder 2005). The insertion of HSE at the 5′-end of any gene may render it heat-inducible (Bienz and Pelham 1986). In fact there are multiple genes in *Drosophila* genomes that contain HSEs and can be induced by HS (Sørensen et al. 2005).

Almost simultaneously in *Drosophila* and in humans a special heat shock transcription factor (HSF) which specifically binds to HSEs after temperature elevation was described. Characteristically, after HS HSF binds predominantly HSE motifs localized in the regions with landmarks of active chromatin which includes histone acetylation, H3K4 trimethylation, the presence of RNA Polymerase II, and coactivators (Guertin and Lis 2010).

There are at least five representatives of HSF family in vertebrates: HSF1, 2, 3, 4 and HSFY (Åkerfelt et al. 2010; Kinoshita et al. 2006; Morimoto 1998; Wu 1995). HSF2 is mainly involved in constitutive Hsps expression and the synthesis of Hsps in embryogenesis and cell differentiation (Åkerfelt et al. 2010; Loones et al. 1997; Wu 1995). The obtained data show that HSF2 and HSF1 can form heterotrimers activating transcription in response to different types of stress and signals during development (Ostling et al. 2007). HSF3 has been originally described in birds and similarly to HSF1 is heat-induced, but only in the case of severe heat shock (Åkerfelt et al. 2010).

Later another transcription factor also designated "HSF3" was described in mice (Fujimoto et al. 2010). However, in mice HSF3 is not able to induce *HSP70* transcription and participates in activation of several "non-canonical" stress-induced genes. HSF4 was cloned from the genomes of mice, rats and humans (Morimoto 1998; Wu 1995). HSF4 is a mammalian factor characterized by its lack of a suppression domain that modulates formation of DNA-binding homotrimer. It was shown that HSF4 gene generates both an activator and a repressor of heat shock genes by alternative splicing. Although both mouse HSF4a and HSF4b form

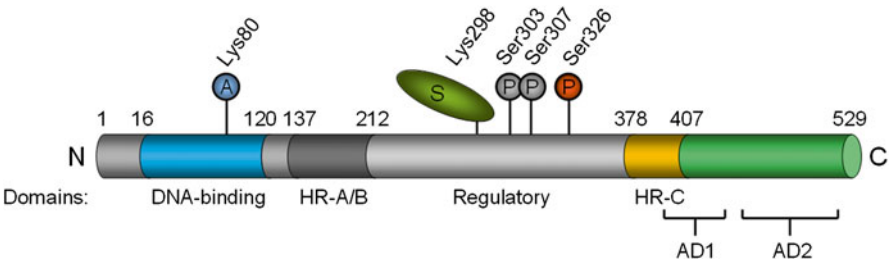

Fig. 3.1 The structure of mammalian HSF1. The relative positions of major structural domains and sites of post-translational modifications are indicated. *AD* activation domains involved in the transcription induction of target genes. HR-A/B and HR-C – hydrophobic repeats responsible for trimerization and downregulation, respectively. Ser303 and Ser307 are phosphorylated under normal non-stressful conditions and participate in downregulation after HS. Lys298 – sumoylation site (S). Phosphorylation of Ser326 plays a key role in activation of HSF1 after trimerization and DNA-binding. Lys80 – undergoes acetylation in the course of HSF1 inactivation (Modified from Åkerfelt et al. 2010 with permission)

trimers in the absence of stress, these two isoforms exhibit different transcriptional activity; HSF4a acts as an inhibitor of the constitutive expression of heat shock genes, and hHSF4b acts as a transcriptional activator. Moreover, heat shock and other stresses stimulate transcription of target genes by HSF4b in mammalian cells (Tanabe et al. 1999).

The fifth mammalian HSF, HSFY, is encoded by gene located in Y chromosome and expresses predominantly in testis. There are data suggesting that deletion of HSFY results in azoospermia syndrome (Shinka et al. 2004).

Interestingly, in *D. melanogaster* only one representative of *hsf* gene family was detected. This gene produces four differently spliced transcripts and participates in the induction of all *Drosophila Hsp* genes (Zimarino et al. 1990). Baker's yeast *Saccharimyces cerevisiae* also comprises only one copy of *hsf* gene (Hahn and Thiele 2004).

Despite low amino acid sequence similarity (usually less than 40 %), heat shock factors of different species share highly conserved secondary and tertiary structures (Morimoto 1998). The structure of mammalian HSF1 is depicted in Fig. 3.1.

DNA-binding domain which recognizes HSE sequences within promoters of heat-inducible genes is located at N-terminal part of HSF. C-terminal end of HSF1 molecule comprises of two domains AD1 and AD2 that modulate the activity of transcription complex (Shi et al. 1995). The 212–407 a. a. region of mammalian HSF1 includes elements responsible for negative regulation of this transcription factor under normal physiological conditions (Fig. 3.1). Specifically, a region between amino acids residues 137 and 212 includes three hydrophobic heptapeptide repeats (designated HR-A/B – hydrophobic repeats A and B) that form leucine zipper motifs responsible for trimerization of HSF1 monomers. The additional leucine zipper is localized within 378–407 a. a. region (HR-C). This fourth leucine zipper (HR-C) is involved in negative regulation of HSF1 by means of intramolecular interaction with the HR-A/B repeats in the absence of stress. Deletion of this

region leads to constitutive trimerization of HSF1 and its transition into active DNA-binding form under normal conditions (Orosz et al. 1996; Rabindran et al. 1993; Westwood and Wu 1993; Zou et al. 1998). Another element which participates in negative regulation of HSF1 is localized within 203–227 region and under normal conditions is able to effectively inhibit activity of transactivation domain (395–503 a. a.) (Morimoto 1998; Rabindran et al. 1993). A region between 300 and 310 a. a. is also involved in negative regulation of HSF1 being a target for constitutive phosphorylation.

The pattern of activation of HSF1 after HS necessary for the initiation of transcription of *Hsp* genes is common for different organisms. The system of heat shock response is in general highly conserved in various phyla. Thus, *D. melanogaster* HSF is able to induce transcription of *HSP* genes in mouse cells where following exposure to stress recruitment and activation of HSF1 occurs (Clos et al. 1993). Under normal conditions HSF in flies and HSF1 in mammals are present in the form of inactive monomers. In mammals inactive HSF1 located mostly in cytosol, while in *Drosophila* HSF at normal conditions reside predominantly in nuclei (Orosz et al. 1996; Wang et al. 2004a; Yao et al. 2006). Inactive HSF1 exists in complex with multiple Hsps as well as many other transcriptional factors regulated by these proteins (see Chap. 2). After temperature elevation these complexes rapidly dissociate and conversion of monomeric form into active trimeric conformation with high-affinity DNA binding capacity occurs in all eukaryotic organisms.

The trimerization results from intermolecular interaction between HR-A/B leucine zippers. Afterwards, in the form of trimers HSF1 is translocated to correspondent chromosomal loci where it binds HSEs (Zimarino et al. 1990; Zou et al. 1998). HSF1 and HSF2 may form heterotrimers that are able to activate transcription in response to variety of stresses and/or at certain stages of development (Ostling et al. 2007). Next, HSF1 undergoes phosphorylation at multiple serine or treonine sites and becomes competent to activate transcription. Interestingly, in baker's yeast *S. cerevisiae* HSF constitutively binds HSEs in the form of trimer under normal conditions due to lack of HR-C leucine zipper. Thus, in *S. cerevisiae* activation of HSF is realized by phosphorylation and interaction with other transcription modulators, because binding of HSF to DNA is required but is not sufficient to activate transcription of heat-induced genes (Bonner et al. 2000; Gallo et al. 1991). It was speculated that *Drosophila* HSF and mammalian HSF1 may itself play a role of thermosensor i.e. its conformational changes leading to trimerization occurs directly as a result of temperature increase. Along these lines, it has been demonstrated that *Drosophila* HSF is constitutively active in human HeLa cells (Clos et al. 1993). It is well known that *Drosophila* dwells at 20–25 °C and, hence, the cultivation temperature of mammalian cells (37 °C) definitely represents stress temperature for the flies cells which should induce *Drosophila* HSF activation and Hsps synthesis. Indeed, it was subsequently demonstrated that xenotransplantation of *D. melanogaster* neuronal cells into mouse brain induces a pronounced synthesis of Hsp70 (Korochkin et al. 2002). On the other hand, the trimerization temperature of human HSF1 in *Drosophila* cells is ten degrees lower than in endogenous (human) cells. It is evident that in the latter case some other factors besides temperature are involved in HSF1 activation.

It was shown that HSF1 trimerization may be triggered at normal temperature by introduction of denatured proteins e.g. BSA into the cell (Ananthan et al. 1986). Besides, activation of HSF1 may be achieved using proteasome inhibitors such as MG132 and clasto-lactacystin β-lactone, leading to dramatic accumulation of poly-ubiquitinated proteins (Pirkkala et al. 2000). According to the modern view under normal (non-stress) conditions HSF1 in human cells exists in complex with Hsp90 dimer, co-chaperone p23 and immunophilin FKBP4 (designated "thermosensitive multichaperoning complex"). Under normal conditions Hsp90 preserves HSF1 in inactive conformation due to intramolecular hydrophobic interactions between leucine zippers HR-A/B and C. After accumulation of denatured proteins in the cytosol Hsp90 begins to interact with them as more highly affinity substrates and, hence, the dissociation of thermosensitive complex and release of HSF1 occurs (Zou et al. 1998). In monomeric state HSF1 is able to rapidly form trimers and bind DNA. Trimerization of HSF1 and transition into DNA-binding form may be also achieved by antibiotic geldanamycin which specifically inhibits Hsp90 activity and induces rapid dissociation of various Hsp90-containing complexes.

Important role of Hsp70 in the negative regulation of HSF under normal conditions was demonstrated in *Drosophila melanogaster* (Solomon et al. 1991). Later physical interaction between *Drosophila* HSF and DroJ (Hsp40) was also shown. Moreover, the DroJ depletion by RNAi technique leads to HSF activation in *Drosophila* cells under normal conditions. Co-depletion of DroJ together with Hsp70/Hsc70 or with Hsp90 leads to the full level induction of the heat shock response. This findings support a model in which synergistic interactions between DroJ1 and Hsp70/Hsc70 and Hsp90 chaperones modulate HSF activity in the course of heat shock response by the negative feed-back interaction (Marchler and Wu 2001).

Thus, in *Drosophila* after temperature elevation Hsp70 is found in all HS puffs in polytene chromosomes where it colocalizes with HSF. It was demonstrated that Hsp70 in cooperation with Hsp40 forms complexes with HSF1 both *in vivo* and *in vitro* (Marchler and Wu 2001). Characteristically, these complexes dissociate after ATP administration. It was further shown that the C-terminal domain of HSF1 is responsible for interaction with Hsp70. Over-expression of Hsp70 placed under the control of constitutive promoter significantly decreases HSF1 activity and inhibits transcription from HSE-containing promoters. Interestingly, in this case HSF1 remains in trimeric form bound to DNA. Therefore, Hsp70 and Hsp40 apparently interfere with the integration of HSF1 into transcription machinery (Baler et al. 1996).

At the stage of recovery after HS, HSF1 interacts with Hsp90 which leads to monomerization and refolding due to intramolecular interaction of leucine zippers A/B and C (Shi et al. 1998). Therefore, accumulation of Hsps after temperature elevation or other challenge inhibits transcription of *Hsp* genes and prevents their own extra accumulation which may be toxic to the cell.

After trimerization HSF1 usually undergoes stress-induced phosphorylation. Several protein kinases including Ca^{2+}/calmodulin-dependent protein kinase (CaMKII), protein kinase C (PKC), DNA-dependent protein kinase (DNA-PK), and possibly cAMP-dependent protein kinase (PKA) are involved in this process.

It was shown along these lines, that CaMKII activation protects cardiomyocytes from HS-induced apoptosis and hypoxia by means of phosphorylation of HSF1 and Hsp70 induction (Peng et al. 2010). Similarly, the application of CaMKII inhibitors such as staurosporine significantly decreases HSF1 activity and heat-inducible accumulation of *HSP70* and *HSP27* mRNA in human glioblastoma cells. On the other hand, the application of PKC agonists such as PMA or ionomycin results in incorporation of (^{32}P) into HSF1 and its hyperactivation (Ding et al. 1997). In yeast protein kinase Snf1 is responsible for inducible HSF phosphorylation under glucose starvation conditions (Hahn and Thiele 2004).

Protein kinases involved in positive regulation of HSF1 activity may be induced by HS due to the increase in concentration of ceramide, cAMP and release of Ca^{2+} ions from endoplasmic reticulum. It is possible that different protein kinases are involved in HSF1 phosphorylation after different kinds of stressful stimuli and the type of protein kinase involved may depend on cell type. There are 12 potential phosphorylation sites within HSF1 molecule located at the following positions: Ser121, Ser230, Ser292, Ser303, Ser307, Ser314, Ser319, Ser326, Ser344, Ser363, Ser419 and Ser444. Phosphorylation of Ser326 probably plays the key role in HSF1 activation after HS and chemical stress (Guettouche et al. 2005; Holmberg et al. 2001; Kiang et al. 1998).

Furthermore, it was demonstrated that different forms of stress activate various phospholipases including neutral and acid sphingomyelinase, phospholipase C, phospholipase A_2 and phospholipase D. Such activation leads to the increase in ceramide C2 concentration due to hydrolysis of sphingomyelin (Nikolova-Karakashian and Rozenova 2010). In *Saccharomices cerevisiae* HS also induces the increase of ceramide level, but in this case, in contrast to mammals, ceramide is synthesized de novo from sphingosine (Wells et al. 1998). The increase of ceramide level may activate SAPK/JNK family of protein kinases and stimulate PKC activity. The increase in ceramide concentration *per se* is not sufficient to induce trimerization of HSF1 or enhance its transcription induction activity. However, elevated level of ceramide significantly extends heat-induced HSF-HSE binding which is necessary for transcription initiation (Nikolova-Karakashian and Rozenova 2010). It is possible to assume that ceramide is one of the most important players at the early stages of HSF1 activation cascade and, hence, while it does not influence the trimerization process it is definitely involved in modulation of phosphorylation system. Therefore, there are two types of early messengers leading to activation of cellular response to different forms of stress. First type represents the appearance of high concentration of denatured proteins resulted in dissociation of HSF1 complexes with chaperones and HSF1 trimerization; second type of messengers includes the increase in concentration of low molecular weight agents such as cermide or cAMP and calcium release from endoplasmic reticulum to the cytosol. These substances trigger protein kinase activation and switching on various regulatory cascades (Nikolova-Karakashian and Rozenova 2010). Low molecular weight mediators, including cAMP serve as important links in many signal pathways including hormone-inducible and, hence, their participation in stress response is not surprising. It is of note, that phosphorylation of different serine residues may have opposite

effect on HSF1 activity. Therefore, while it is known that activation of HSF1 requires phosphorylation of Ser326, phosphorylation of Ser303 and Ser307 leads to inactivation of HSF1 and represents an obligatory step of negative regulation of heat shock response. Furthermore, phosphorylation at Ser303 and Ser307 residues takes place in the course of recovery process after HS and is accomplished by mitogen-activated/extracellular signal regulated protein kinases (MAPK/ERK), p38/JNK (c-jun N-terminal kinase) and glycogen synthase kinase-3β (GSK-3). Stress-induced protein kinases p38 and JNK activity may lead to the death of cells by apoptosis after severe stress by means of HSF1 inactivation and blocking *Hsp* genes induction (Anckar and Sistonen 2007; Chu et al. 1996; He et al. 1998).

It is of note that there is strict hierarchy in phosphorylation of different serine residues within HSF1 molecule. Thus, phosphorylation of Ser303 occurs only after phosphorylation of Ser307. Negative influence of Ser303 phosphorylation on HSF1 activity is based on two mechanisms. First, HSF1 phosphorylated at Ser303 position binds with protein 14-3-3epsilon which facilitates its transport from nucleus to cytoplasm (Wang et al. 2004b). Second, phosphorylation at Ser303 residue serves as a signal for sumoylation of HSF1 at lysine-298.

Proteins belonging to SUMO family (SUMO1 and SUMO2/3) are also involved in HSF1 regulation. SUMO family (Small Ubiquitin-related Modifier) comprised several ubiquitin-like proteins that interact with their substrates by means of isopeptide bonds and are involved in the regulation of multiple transcriptional factors. It was shown that SUMO1 binding effectively inhibits transcriptional activity of both HSF1 and HSF2 (Anckar and Sistonen 2007). Similarly, SUMO2/3 proteins are also able to inhibit HSF1 activity. Interestingly, modulation of HSF1 activity by means of SUMO2/3 requires HSP27 which, thus, may modulate the expression of its own genes by negative feedback after excessive accumulation in the cell (Simioni et al. 2009).

In addition to 14-3-3 epsilon and SUMO2/3 oligomeric form of HSF1 specifically binds to HSBP1 (HSF-binding protein 1) which interacts with oligomerization domain of HSF1 (leucine zippers A/B) and inhibits its activity. Thus, over-expression of HSBP1 in C. *elegans* cells renders them less resistant to HS and arsenite (Cotto and Morimoto 1999). Apart from phosphorylation, HSF1 activity is regulated via acetylation/deacetylation of DNA-binding domain. In fact, acetylation of lysine-80 results in the loss of DNA-binding ability of HSF1 which represents one of the important steps in negative regulation of HSF1 activity in the course of heat shock response (Westerheide et al. 2009). General scheme of HSF1 regulation is depicted in Fig. 3.2.

Studies performed on *Drosophila* contributed significantly to the understanding of mechanisms underlying eukaryotic *Hsp* genes and in particular *Hsp70* activation. It was shown that the basic transcriptional machinery is pre-assembled at the stress-responsive genes under non-stress conditions, and it is the binding of HSF1 and its phosphorylation at the promoters that leads to rapid induction of stress-inducible genes expression. Specifically, in *D. melanogaster* cells RNAP II is constitutively bound to the promoters regions of *Hsp70* and *shsps* genes at −12…+65 bp position in relation to the transcription start (Belikov and Karpov 1996; Lis 2007).

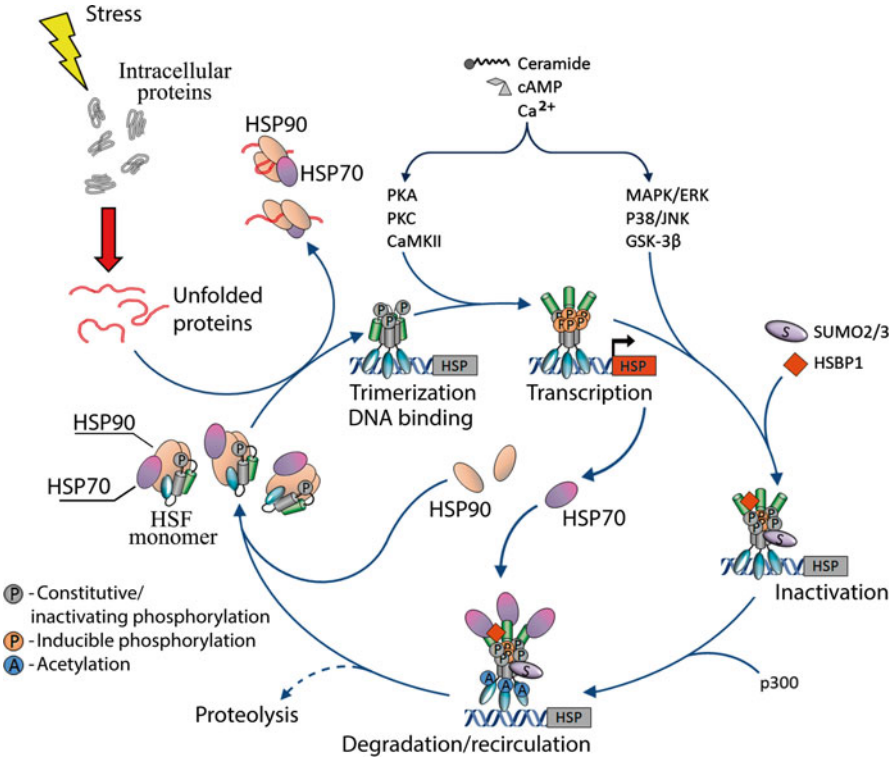

Fig. 3.2 Activation and downregulation cycle of HSF1. Under non-stress conditions HSF1 monomers form complexes with Hsp90 and (as was shown in *Drosophila*) Hsp70. The appearance of large quantities of denatured proteins leads to the dissociation of HSF1 complexes with Hsp90 and its trimerization. Such trimers bind to DNA, but do not induce transcription. At the next stage HSF1 is activated by phosphorylation which renders it effective transcription inducer of the heat shock genes. In turn, phosphorylation at Ser303 and Ser307 residues, triggers sumoylation and as a result subsequent inactivation of HSF1. Stress-induced protein kinases involved in HSF1 phosphorylation are activated by elevated cytosolic levels of ceramide, cAMP and Ca^{2+} ions. HSF1 also interacts with HSBP1 protein, which inhibits its activity. After this HSF1 form complex with Hsp70 which does not influences its DNA-binding activity but effectively excludes HSF1 from transcription machinery. Finally, HSF1 loses its DNA-binding activity due to acetylation and binds to Hsp90 acquiring monomeric inactive form. In certain cases inactivated HSF1 may be subjected to proteolysis (Åkerfelt et al. 2010; Anckar and Sistonen 2007; Belikov and Karpov 1996; Lee et al. 1992; Peng et al. 2010; Zhang et al. 1998, 1999)

The packaging of DNA into nucleosomes affects all phases of the transcription cycle. Specifically, recruitment of chromatin remodeling factors to *hsp* genes plays a key role in their rapid induction by providing transcription factor accessibility at stress-responsive genes promoters (Guertin and Lis 2010; 2013). It was demonstrated that 5′-end of *Drosophila Hsp70* genes is devoid of histone H1 under non-stress conditions and contains a nucleosome-free region that extends further downstream (Karpov et al. 1984; Shopland et al. 1995; Tsukiyama et al. 1994). In response to HS chromatin architecture throughout *D. melanogaster Hsp70* genes

undergoes an initial dramatic change, a change that does not depend on transcription, followed by a second disruption of nucleosome structure that is transcription-dependent (Petesch and Lis 2008).

The widespread changes in chromatin structure at *Hsp70*-containing region following HS also critically dependent on poly (ADP-ribose) polymerase (PARP). PARP has been previously identified as a critical factor for polytene chromosome puffing in response to HS. Catalytic inhibition of PARP results in the failure of *Hsp70* to undergo rapid transcription-independent loss of the chromatin structure following HS (Petesch and Lis 2008). These data suggest that production of long nucleic acid-like poly(ADP-ribose) (PAR) molecules may result in a histone loss and chromatin decondensation throughout the whole length of transcribed DNA, the event that facilitates the movement of RNAP II along DNA (Guertin et al. 2010; Petesch and Lis 2012).

These peculiarities of regulatory regions facilitate extremely rapid response of *Hsp* genes transcription machinery to HS and other forms of stress. Thus, *Hsp* mRNA in *Drosophila* cells is detectable within 15–30 s after HS. In *D. melanogaster* the product of *trl* gene plays important role in supporting the open chromatin structure and RNAP II positioning on the *Hsp70* and certain other *Hsp* promoters. This protein designated GAGA-binding factor (GAF) is able to bind with GA/CT repeats (Omelina et al. 2011). *Drosophila Hsp70* promoters contain several regularly spaced GAF-binding sites within 150 bps upstream of transcription start site in direct (GAGAGAG) and inverse (CTCTCTC) orientations (Wilkins and Lis 1997; Georgel 2005). Binding GAF to *Hsp70* promoter triggers modification of histone H3 throughout the whole *Hsp70* genes transcribed region and results in chromatin decompactization. It is assumed that insulators scs (specialized chromatin structures) and scs' represent boundaries of DNA decompactization in *Hsp70* gene cluster in *Drosophila* (Hart et al. 1997; Petesch and Lis 2008). More details concerning the functions of these insulators are provided in Chap. 5.

Apart from chromatin modification, GAF is required for interaction of RNAP II with transcription initiation region in complex with general transcription factors (GTFs) such as TBP (TATA-box binding protein). Interestingly, other general transcription factors from TFII family are present in the promoters of *Hsp70* genes under non-stress conditions but dissociate after temperature elevation (Lebedeva et al. 2005). In the case of *Drosophila* in non-stressed cell *Hsp* genes are already bound by RNAP II, but the polymerase is paused after transcribing 20–40 nucleotides. The paused polymerase is associated with NELF (negative elongation factor) and Spt4/5 (or DRB sensitivity inducing factor, DSIF), that effectively inhibit transcription elongation. Following temperature elevation or other challenge, escape of the paused polymerase requires recruitment and activation of HSF resulting in dissociation of NELF. At the next stage the large subunit of RNAP II is phosphorylated at C-terminal domain (CTD) by positive transcription elongation factor PTEFb (Lee et al. 2008a, b; Wu et al. 2003) to activate transcription. PTEFb also phosphorylates negative elongation factor (NELF) and transcription elongation factor Spt5, thus releasing polymerase into productive transcription elongation (Fig. 3.3). Interestingly, HSF does not directly interact with RNAP II rather acts through the

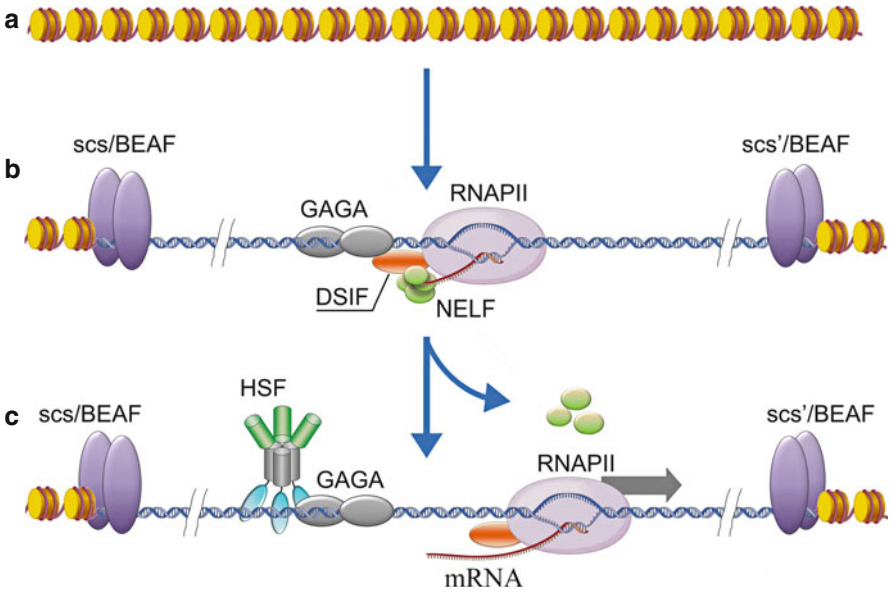

Fig. 3.3 Role of GAGA-factor (GAF) in transcription activation of *D. melanogaster Hsp70* genes. Interaction of GAF with promoters results in chromatin decondensation in *Hsp70* genes region providing conditions for the formation and positioning of RNAP II preinitiation complex. In non-stressed cells after initiation RNAP II interacts with DSIF and NELF factors and is paused after transcribing 20–40 nucleotides (**a**, **b**). Afterwards, RNAP II bound with short transcript and DSIF and NELF factors remains in the transcriptional pausing state until binding of HSF takes place. Interaction of HSF with transcriptional complex results in dissociation of NELF and transition of RNAP II into elongation stage (**c**). scs/scs′ elements bound with BEAF32 protein serve as boundaries of decondensed active chromatin (Modified from Hart et al. 1997; Petesch and Lis 2008; Wu et al. 2003)

mediator complex (Park et al. 2001). It is of note, that the described mechanism of *Hsp70* genes activation is not universal even for different *Drosophila* heat shock genes. For instance, promoters of *Hsp68* и *Hsp83* genes do not contain GAGA motifs and, hence, do not interact with GAF. However, the transcription of these genes is strongly induced by HS and they form large puffs in polytene chromosomes after temperature elevation and other forms of stress.

In mammals under normal temperature conditions CHBF protein (constitutive HSE-binding factor) or Ku-autoantigen is bound to the promoter of *HSP70* (Kim et al. 1995; Tang et al. 2001; Turturici et al. 2009; Yang et al. 1996). This protein exists in heterodimeric form consisting of two subunits with molecular masses 70 and 86 kD, respectively. Ku-protein was originally described as transcriptional factor for RNA Polymerase III involved in tRNA synthesis. Additionally, CHBF (Ku-protein) plays important role in DNA reparation system and in the process of V(D)J-recombination (Nussenzweig et al. 1996). In the case of *HSP70* regulation, Ku interacts with Sp1 and GAGA-binding protein, and may play a significant role in the *HSP70* constitutive expression (Turturici et al. 2009). In *Drosophila* YPF1

(Yolk protein factor 1) is orthologues to the mammalian Ku-protein and binds specifically to *Hsp70* promoters in non-stress conditions (Jacoby and Wensink 1994). Heat shock alleviates this interaction and CHBF is partially substituted by activated HSF1. The reverse process occurs during recovery from HS.

Certain chemical agents such as arsenite or kadmium chlorid may induce the transcription of constitutive member of *Hsp70* family, namely *Hsc70*. However, after such treatment inducible Hsp70 may not be expressed and CHBF may remain bound to HSE. Similarly, while mild HS (41 °C) in mammalian cells induces HSF1 activation the expression of Hsp70 is not increased and CHBF remains bound to the promoter. Only after acute HS the expression of *HSP70* genes is dramatically activated and CHBF is released from promoter and superseded from transcriptional complex by HSF1 (Yang et al. 1996).

In 2005 a new factor termed "menin" involved in *D. melanogaster hsp* genes regulation has been described (Papaconstantinou et al. 2005). It was shown that menin belongs to stress-inducible factors and binds to *Hsp* genes promoters after HS. Inactivation of menin results in the time decrease of *Hsp70* and *Hsp23* transcription after HS, while over-expression of menin significantly prolonged *Hsp* transcription period after HS and during the recovery period.

As we mentioned above, in different organisms there are other factors involved in *Hsp* genes transcription regulation besides HSF1. Thus, small heat shock genes in *Drosophila* may be induced by molting hormone ecdysone (Cheney and Shearn 1983; Thomas and Lengyel 1986). In mammalian cells Sp1 protein as well as CBP (CCAAT-binding protein) and CTF (CCAAT-box-binding transcription factor) binds to promoters of *HSP70* genes providing their constitutive transcription (Bevilacqua et al. 1997; Morgan et al. 1987). Interestingly in mammals promoters of *HSP70A1A*, *HSP70A1B* and *HSP90* genes contain recognition sites for NF-kB and STAT-3. These proteins can regulate heat-shock genes in response to cytokine (TNF and interferon-γ) stimulation. Furthermore, NF-IL6 and HSF1 interacts with each other as antagonists (Stephanou et al. 1999; Tang et al. 2001).

Recently group of Evgeny Nudler described in mammalian cells long non-coding RNA termed "HSR1" (heat shock RNA-1) which according to their experiments serves as thermosensor and represents another key determinant in this process (Shamovsky et al. 2006; Shamovsky and Nudler 2009). They showed that *in vitro* HSR1 forms a complex with eEF1A (eukaryotic elongation factor 1A), which is required for HSF1 trimerization and its subsequent DNA binding (reviewed by Place and Noonan 2014). Unfortunately, HSR1 gene was not found in the genome of any mammalian species yet.

The system comprised of HSF and associated factors responsible for activation of "classical" heat shock genes is triggered predominantly by increased concentration of denatured proteins in the cytosol. Besides temperature elevation and other non-specific stressful stimuli influencing all cellular systems, there are other forms of stress (e.g. administration of inhibitors of N-glycosylation) that act selectively at the level of protein folding in endoplasmic reticulum (ER). Therefore, cells need to specifically control the state of proteins in endoplasmic reticulum. Indeed, such system designated "UPR" (unfolded protein response) exists and effectively

functions (Chen and Brandizzi 2013). UPR system includes several chaperones (ER-chaperones) translocated to endoplasmic reticulum after HS. The synthesis of this class of chaperones represents adaptive reaction at the cellular level in response to abnormal protein folding in the ER.

On the other hand activation of UPR system may induce cell death by apoptosis in the case of severe stress which cannot be compensated by the chaperones protective activity (Chen and Brandizzi 2013).

Like HSF-regulated response, UPR system is rather conserved. Ire1 protein (inositol-requiring enzyme 1), represents the basic element of this system. In different organisms including yeasts, mammals and plants Ire1 serves as receptor able to sensor stressful stimuli at the level of endoplasmic reticulum and activate transcription of the target genes. Ire1 is a typical transmembrane protein localized in the endoplasmic reticulum membrane and comprised of three functional domains: stress-sensory intraluminal domain which serves as receptor and two catalytic domains of cytosolic localization, responsible for protein kinase and endoribonuclease activities, respectively (Fig. 3.4). In mammals there are two isoforms of Ire1 – IRE1α and IRE1β. Under non-stress conditions IRE1α is blocked by interaction of intraluminal domain with HSPA5/BiP. After stress IRE1α and BiP dissociate from the complex because misfolded or denatured proteins that appear in endoplasmic reticulum have higher affinity to BiP.

Activation of HSF and UPR-systems in mammalian cells shares several important features. In both cases chaperones play a role of negative regulators under non-stress conditions and form stress-sensory complex with major transcription factor (HSF1 or Ire1). This complex readily responds to the accumulation of denatured proteins. On the other hand in the case of Ire1 in yeast and IRE1β in mammals activation is driven by direct interaction with denatured proteins, and is independent on BiP dissociation from the complex (Chen and Brandizzi 2013). Following activation, Ire1 oligomerizes and undergoes autophosphorylation leading to drastic conformation change and activation of endoribonuclease domain. Subsequently, endoribonuclease performs noncanonical splicing of the mRNA encoding factor, responsible for transcription of UPR target genes, described in yeast, plants, *Drosophila* and mammals, as Hac1, bZIP60 and Xbp-1, respectively. Under normal conditions mRNA of these genes is localized in cytosol in inactive state. After UPR-system activation the intron containing translation attenuator is cleaved out from the target mRNA by Ire1 endoribonuclease (Mori et al. 1996, 1998). Therefore, after splicing Hac1/bZIP60/Xbp-1 mRNA may be efficiently translated. Synthesized protein is then translocated into the nucleus and binds to regulatory regions of target genes drastically enhancing their transcription efficiency (Fig. 3.4). In yeast Hac1 recognizes specific nucleotide motif designated UPRE (unfolded protein response element). The sequence represents imperfect palindrome with 1 bp spacer: CAGCGTC (Mori et al. 1998). In yeast UPRE-like elements are involved in regulation of seven genes including *Kar2*, *Lhs1* and *Cer1p* that belong to *Hsp70* family. These genes (e.g. *Kar2*) may contain HSE motifs within promoter region and, hence, be regulated by HSF (Foti et al. 1999). On the other hand, yeast HSF as well as Hac1 may be activated through phosphorylation by protein kinase Snf1 in

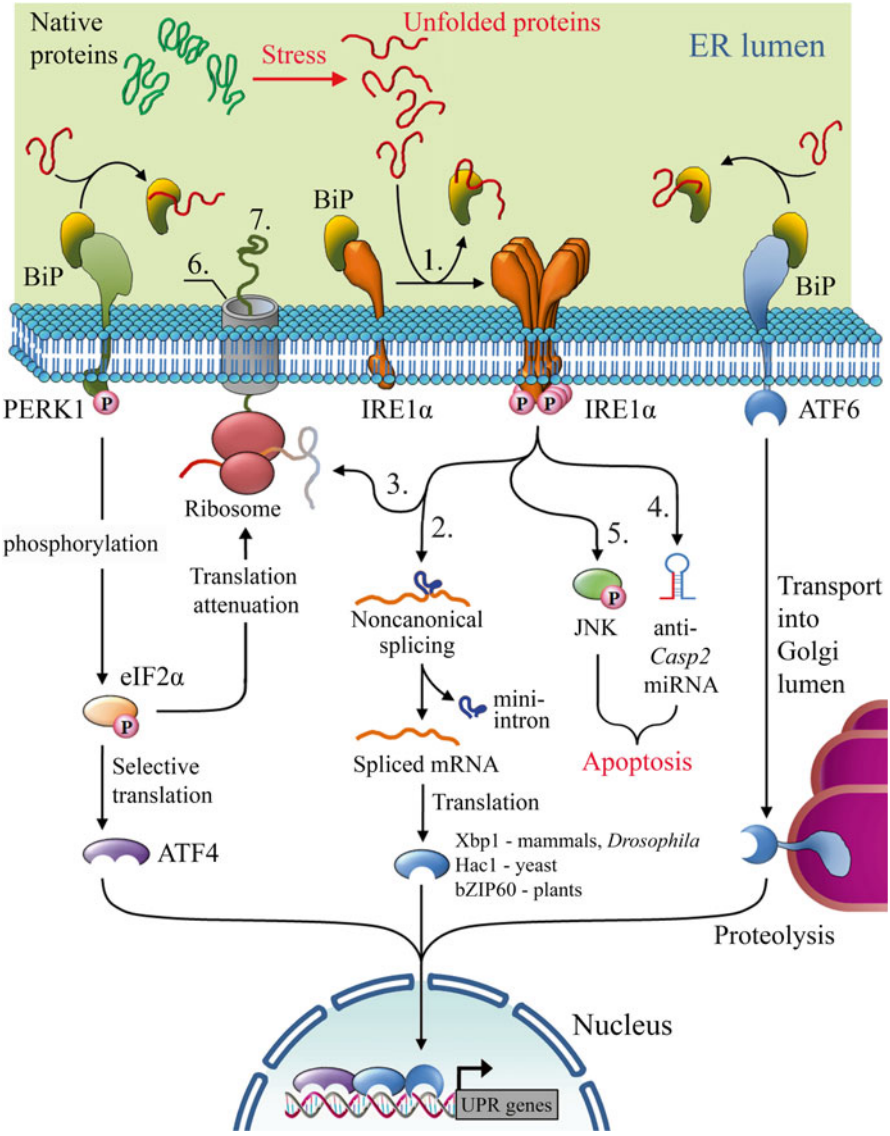

Fig. 3.4 General scheme of UPR regulation. *1* Appearance of unfolded proteins as a result of the ER stress leads to the Ire1α-BiP complex dissociation and Ire1α oligomerization. After this Ire1α autophosphorylation and activation occurs, and Ire1α induces a few signal cascades: *2* noncanonical splicing of mRNAs coding Xbp1/Hac1 transcriptional factors; *3* ER-proteins mRNAs cleavage leads to the block of the translocation of certain proteins into ER and subsequent apoptosis in the case of severe stress; *4* and *5* proapoptotic signalling through antiapoptotic miRNA cleavage and phosphorylation and activation of JNK. Translocone and nascent protein translocated into ER are denoted in this scheme as *6* and *7* respectively. *ER* endoplasmic reticulum, *BiP* binding immunoglobulin protein, *JNK* c-jun N-terminal kinase (Modified from Lee 2001; Chen and Brandizzi 2013)

response to ER-stress stimuli, e.g. glucose deprivation (Hahn and Thiele 2004). In mammals, besides, Xbp-1 there are several other transcriptional factors such as ATF4 and ATF6, as well as NF-Y, YY1 and YB-1 involved in regulation of ER-chaperones (Li et al. 1997; Roy et al. 1996). In order to respond to UPR activation promoters of target genes in mammals should contain two alternative sequences designated ERSE and ERSE-II (ER stress element). ERSE represents three structure unit 5′-CCAAT(N_9)CCACG-3′, where N_9 – GC-rich region with consensus CGGCGGCGG (Foti et al. 1999; Lee 2001; Roy and Lee 1999). In humans promoter of *HSPA5* gene contains three ERSE elements spaced at −126, −94 and −61 bps upstream of TATA-box. Promoter of *GRP94* gene contains two ERSE in inverse orientation located at −195 and −137 bps upstream the transcription start site and the third ERSE at -72 bps. ERSE-II (ATTGG(N)CCACG) was found in the promoter of *Herp* and several other genes (Lee 2001).

Apart from splicing of Xbp-1 mRNA in *Drosophila* and mammals Ire1 cleaves various RNA preventing the translation of certain proteins transported into endoplasmic reticulum by means of recognizing consensus sequences responsible for endoplasmic localization of the proteins (Regulated IRE1-Dependent Decay, or RIDD). Thus, Ire1 activity blocks the translocation of certain proteins into ER until the conditions necessary for normal folding of the proteins in ER are restored. Extremely severe stress instead of adaptive response may induce apoptosis due to cleavage by Ire1 of a few anti-apoptotic pre-mRNAs involved in suppression of caspase-2 (Chen and Brandizzi 2013).

In contrast to yeast, in mammals besides Ire1 two other factors (PERK and ATF6) are involved in regulation of UPR-system (Fig. 3.4). Similarly to Ire1, they represent typical transmembrane proteins activated by stress factors interfering with normal function of endoplasmatic reticulum. PERK (RNA-dependent protein kinase-like ER kinase) is a protein kinase which attenuates translation by phosphorylation of eIF2α, one of the key translation initiation factors. The decline of translation level as well as cleavage of certain mRNAs by Ire1 leads to general decrease in the amount of proteins transported to ER subjected to denaturation and aggregation and, hence, facilitate the functioning of chaperonic machinery under stress conditions. On the other hand, phosphorylated eIF2α initiates translation of a small specific subpopulation of mRNAs containing small upstream open reading frames (suORFs) in its 5′-leader region including ATF4 (activating transcription factor 4) which in turn activates the transcription of certain UPR target-genes (Harding et al. 2000). The third ER-stress response factor is ATF6 (activating transcription factor 6). When ATF6 is activated it is translocated into Golgi complex where it undergoes specific proteolisis leading to cleavage of cytosolic domain. This domain is transported into the nuclei, where it binds regulatory sequences in dimeric form and activates the target genes (Fig. 3.4) (Chen and Brandizzi 2013). It is important to note that PERK and ATF6 are activated only after their dissociation from complex with BiP (de la Cadena et al. 2013).

After heat shock and several other forms of stress in parallel with dramatic activation of *Hsp* genes battery, the repression of virtually all other genes takes place. This is clearly illustrated by a rapid regression of all non-heat shock inducible

puffs in *Drosophila* polytene chromosomes after HS. Ribosomal and histone genes remain transcriptionally active after HS (although their splicing is inhibited) as well as tRNA loci and genes encoded by mitochondrial genome (Bonner and Pardue 1977; Bonner et al. 1978). Temperature elevation results in rapid disruption of old polysomes and formation of new ones where Hsps are predominantly synthesized. The disruption of old polysomes occurs not as a result of old and newly synthesized mRNA competition. Thus, if simultaneously with the temperature increase α-amanitin or actinomycin D is added to the cells, new mRNAs would not be synthesized, but the old polysomes, however, would be mostly destroyed. Some pre-HS polysomes are stored in the cytoplasm but their translation is blocked. When the temperature returns to normal the synthesis of normal cellular proteins resumes on these preexisting polysomes (Kruger and Benecke 1981).

Taken together, temperature elevation and other forms of stress not only represses the transcription of most genetic loci but also blocks the translation of the mRNAs already present in cytoplasm, decreasing the amount of proteins that may be damaged by stressful stimuli. On the other hand strong induction of Hsps synthesis is definitely of protective nature and serves to protect the genetic apparatus of the cell from harmful stress consequences.

Selective transcription of *HSP* genes in mammalian cells after stress is provided by means of drastic changes in phosphorylation kinetics of the C-terminal domain (CTD) of the large subunit of RNAP II. It is known that during transcription RNAP II CTD phosphorylation occurs at serine and treonine residues. Furthermore, dephosphorylated form of CTD is involved in the formation of transcription initiation complex while the transition to elongation stage requires phosphorylation of CTD. Several CTD-specific protein kinases including DNA-PK and MAPK as well as CTD-specific phosphatase FCP-1 are involved in the dynamic process of CTD phosphorylation/dephosphorylation. It was demonstrated in HeLa cells that specific stress-CTD-kinases (e.g. DNA-PK and MAPK) are responsible for CTD hiperphosphorylation during the HS. It is of note, that CTD-kinases that function in the cells under non-stress conditions are inactivated by temperature elevation and other forms of stress. Simultaneously, inactivation and denaturation of FCP-1 takes place (Dubois et al. 1999; Venetianer et al. 1995). It is likely that hyperphosphorylation of CTD results in the loss of RNAP II affinity to the promoters of non-heat shock genes and, hence, leads to drastic increase of *Hsp* genes transcription.

The second mechanism by which heat shock suppresses gene transcription is through the upregulation of inhibitory non-coding RNAs that block general RNAP II activity. Two non-coding RNA species including B2 RNA in mice and Alu RNA in humans are transcribed by RNA polymerase III from short interspersed elements (SINEs). During normal cellular growth, these RNA species accumulate at relatively low levels; however, their abundance transiently increases by as much as 40-fold under stress conditions (i.e. heat shock). There is no shared sequence homology between B2 and Alu RNAs. However, their biological functions are considered to be very similar if not identical. Following stress, B2 or Alu RNAs directly bind to the active site of RNAP II, disrupting its interaction with promoter DNA and interfering with CTD phosphorylation (Place and Noonan 2014).

It was found in *Drosophila* that after HS activated HSF also binds to promoters of a large set of genes that are not transcriptionally induced by temperature elevation. Characteristically, the promoters of these genes are usually enriched with the insulator protein, BEAF prior to HS (Gonsalves et al. 2011). It was hypothesized that *Drosophila* HSF might play a role in the transcriptional repression of certain genes that are known to be inactivated during HS (Westwood et al. 1991). HSF binding sites were also found in introns of certain genes involved in developmental processes and reproduction. For example, HSF during HS binds to introns of the three major ecdysone-inducible genes, and HSF binding with these genes is coincident with their repression. Therefore, HSF may play a direct role in the repression of these genes (Gonsalves et al. 2011).

Along these lines, it was demonstrated that in mammalian cells HSF1 may repress the transcription of TNFα gene by binding to its 5'-UTR region (Singh et al. 2002).

Importantly, HS effectively inhibits splicing of normal cellular pre-mRNAs. It is of note, that inducible *Hsp* genes in most organisms are intronless and, hence, do not require splicing for normal function. It was shown that high molecular weight Hsps such as Hsp90 and Hsp70 in cooperation with Hsp40 are involved in restoration of normal splicing process after stress termination (Vogel et al. 1995). Interestingly, in organisms with intron-exon arrangement of *Hsp* genes (e.g. *Trypanosoma*, *C. elegans*), splicing of *Hsp70* mRNA occurs with maximal efficacy after moderate HS while splicing of "normal" cellular genes such as tubulin is concomitantly blocked by not yet described mechanism (Huang and Van der Ploeg 1991).

Furthermore, HS and certain viral infections severely inhibit the translation of most cellular mRNAs due to drastic changes in the translation initiation complex. The mechanisms underlying translation inhibition by HS and during viral infection share many features. As a result, in both cases strong activation of double stranded RNA-activated protein kinase (PKR) is usually observed. Furthermore, hypoxia and HS leads to activation of protein kinase HRI (heme regulated inhibitor of translation), while the disturbance of ER functions by stress activate protein kinase PERK. Both protein kinases phosphorylate α-subunit of eukaryotic translation initiation factor eIF2 at serine-51 (Sheikh and Fornace 1999). After phosphorylation eIF2 is incapable to perform the GTP-GDP exchange in complex with eIF2B factor, and, hence, the blockade of translation initiation occurs. The degree of eIF2α phosphorylation is increased two to three folds after temperature elevation (Duncan et al. 1995; Gallie et al. 1997; Menon and Thomason 1995).

Dephosphorylation of eIF4E-bound proteins including eIF4E-BP1, eIF4E-BP2 and eIF4E-BP3 represents the second mechanism of translation inhibition after stress including HS. Dephosphorylation blocks the interaction of cap-recognizing factor eIF4E with eIF4G (but not with cap itself). Dephosphorylated form of eIF4E-BP exhibits high affinity to eIF4E and its binding results in general decrease of total translation level in the cell (Vries et al. 1997). Heat shock and viral infection induce dephosphorylation of eIF4E, decreasing its affinity to mRNA 5′-cap. It was also demonstrated that Hsp27 inhibits the activity of eIF4G. *In vitro* and *in vivo* experiments revealed their direct interaction. It was shown that Hsp27 binds to

eIF4G and transforms it into insoluble state. In contrast, Hsp70 initiates the reverse process leading to the restoration of eIF4G activity (Cuesta et al. 2000).

After HS or viral infection concomitant to substantial drop in translation efficiency (up to ten fold) in the affected cells selective translation of viral or *Hsp* mRNAs proceeds. Such selectivity is provided (among other reasons) due to the presence of specific motifs within the sequences of viral or *Hsp* RNAs. The characteristic differences at the 5′-UTRs of mRNAs of heat shock genes and "normal" cellular genes transcribed under non-stress conditions were detected soon after the discovery of HS response at the molecular level. Thus, in most organisms including *Drosophila* species and various mammals, *hsp70* mRNA contains 250 bps 5′-leader consisting predominantly of purines. Different lines of evidence suggest that translation of *HSP70* mRNA in humans after HS resembles translation of picornaviruses or late adenoviruses mRNAs. It is generally assumed that after HS *HSP70* mRNA is translated exploring mechanism designated by different author as "jumping", "shunting" or "hopping" (Yueh and Schneider 2000). According to the assumed scheme, in the process of scanning of the 5′-leader, ribosomal 40S subunit "jumps" over certain region. Such a mechanism is determined by the secondary structure of 5′-UTR leader of *HSP70* mRNA. Thus, under normal conditions 40S ribosome may scan the whole length of 5′-leader while after HS or viral infection the translation of specific mRNAs containing certain structure in their leaders occurs by "jumping" mechanism. This mechanism does not require a complete assembly of translation initiation complex, hence, enabling *HSP70* or adenovirus mRNA to be selectively translated. 5′-UTR of adenoviral mRNA (Ad5) contains three regions (C1, C2 and C3, respectively) exhibiting strict complementation with two regions of the hairpin located at the 3′-end of 18S RNA from 40S ribosome subunit. The deletion of both C2 and C3 regions results in 20 fold drop in translation efficacy corroborating their direct involvement in the process. Similarly, 5′-UTR of mammalian *HSP70* mRNA contains a region complementary to 3′-hairpin of 18S rRNA and the deletion of this particular region decreased the translation after 44 °C HS more than three times. Molecular mechanism underlying initiation in the case of *HSP70* and adenoviral mRNAs is not fully understood yet, but possibly resembles that of prokaryotes which requires Shine-Dalgarno sequence (Yueh and Schneider 2000).

According to other authors (Hernández et al. 2004; Rubtsova et al. 2003) *HSP70* mRNA is translated through a well-known mechanism described for many viral mRNAs. This mechanism is based on internal initiation due to the presence of IRES sequences (Internal Ribosome Entry Site). IRES binds to preinitiation complex together with 40S ribosome subunit and eIF2 and eIF3 factors and positioned mRNA start codon exploring cap-independent mechanism missing preliminary scanning of 5′-UTRs with involvement of eIF4G and eIF4A factors. Interestingly, the addition of *HSP70* 5′-UTR to a reporter construct enhances its translation efficiency more than 100 fold which is close to the effect of classical picornavirus IRES (Rubtsova et al. 2003).

In this way, heat shock causes inactivation of the cap-binding initiation factor eIF4E and drastically reduces the abundance of the both components and assembled translational initiation complexes. Under these conditions, rapid inhibition of

normal cellular mRNAs translation occurs, while *Hsp70* mRNA is selectively translated by cap-independent mechanism.

Recently it was found using mouse and human cells that severe heat stress triggers global pausing of translation elongation of most cellular mRNAs at around 65 codon. It was also demonstrated that severe HS reduces HSC70/HSP70 association with ribosomes and alters interactions with the translational machinery. Preferably pausing occurs on nascent peptides with hydrophobicity of the N-termini which binds with Hsp70. In the absence of ribosome-associated Hsp70 during heat stress, nascent peptides with stronger Hsp70 binding motifs might have a greater tendency to misfold or aggregate, potentially impacting the efficiency of translation. Hsp70 overexpression or mild preliminary heat shock protects cells from heat shock-induced elongation pausing. Elongation pausing represents an important component of cellular stress responses (Shalgi et al. 2013).

It is of note, that although the details of heat shock genes activation and functioning attracted a lot of attention (see above) the whole picture is far from being clear. Thus, recently Prof. Nudler and his colleagues demonstrated that in mammals translation elongation factor eEF1A1 which has a well-defined role in the protein-synthesis machinery also functions as an essential regulator of human HSPs. This factor was shown to participate in each major step of Hsps induction. Upon stress, nuclear pool of eEF1A1 increased dramatically and the protein activates transcription by recruiting HSF1 to *Hsp* promoters. Subsequently, this factor associates with 3′UTRs of HSP mRNAs, stabilizing them and facilitating their transfer from the nucleus to the ribosomes. Interestingly, prior to stress, eEF1A1 is required for *Hsp* gene silencing (Vera et al. 2014).

3.1 Conclusions

Heat shock response system is highly conserved in all eukaryotes and comprises the same regulatory features practically in all organisms studied. Different forms of stress cause accumulation of misfolded proteins and a few specific low-molecular messengers (such as ceramide) in the cell. Misfolded proteins interact with molecular chaperones as high-specific substrates which results in dissociation of the temperature sensitive complex between certain Hsps and HSF, the major stress transcription factor. After that HSF forms homotrimers, which specifically recognize heat shock regulatory elements (HSEs) within promoters of *Hsp* genes and activate transcription. After Hsps accumulation following heat shock they bind and inactivate HSF by negative feed-back mechanism. Besides general transcription factors there are multiple coactivators of HSR that participate in modifications of histones and subsequent decompactization and remodeling of chromatin landscape which determines HSF binding at the regions of heat-induced transcription. Besides HSF, there are many other proteins involved in heat-induced transcription and translation of *Hsp* genes (e.g. eEF1A1). Thus, HS response represents a complex multilevel system, very flexible in terms of the ability to rapidly react to a variety of environmental and metabolic stresses.

References

Åkerfelt M, Morimoto RI, Sistonen L (2010) Heat shock factors: integrators of cell stress, development and lifespan. Nat Rev Mol Cell Biol 11:545–555

Amin J, Nestril R, Schiller P, Dreano M, Voellmy R (1987) Organization of the Drosophila melanogaster hsp70 heat shock regulation unit. Mol Cell Biol 7:1055–1062

Amin J, Ananthan J, Voellmy R (1988) Key features of heat shock regulatory elements. Mol Cell Biol 8:3761–3769

Amin J, Fernandez M, Ananthan J, Lis JT, 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

Ananthan J, Goldberg AL, Voellmy R (1986) Abnormal proteins serve as eukaryotic stress signals and trigger the activation of heat shock genes. Science 232:522–524

Anckar J, Sistonen L (2007) Heat shock factor 1 as a coordinator of stress and developmental pathways. Adv Exp Med Biol 594:78–88

Baler R, Zou J, Voellmy R (1996) Evidence for a role of Hsp70 in the regulation of the heat shock response in mammalian cells. Cell Stress Chaperones 1:33–39

Belikov SV, Karpov VL (1996) Mapping protein-DNA interaction with CIS-DDP: chromatine structure of promoter region of D. melanogaster HSP70 gene. Biochem Mol Biol Int 38:997–1002

Bevilacqua A, Fiorenza MT, Mangia F (1997) Developmental activation of an episomic HSP70 gene promoter in two-cells mouse embryos by transcription factor Sp1. Nucleic Acids Res 25:1333–1338

Bienz M, Pelham HRB (1986) Heat shock regulatory elements function as an inducible enhancer in the Xenopus hsp70 gene and when linked to a heterologous promoter. Cell 45:753–760

Bonner JJ, Pardue ML (1977) Polytene chromosome puffing and in situ hybridization measure different aspects of RNA metabolism. Cell 12:227–234

Bonner JJ, Berninger M, Pardue ML (1978) Transcription of polytene chromosomes and of the mitochondrial genome in Drosophila melanogaster. Cold Spring Harb Symp Quant Biol 42:803–814

Bonner JJ, Carlson T, Fackenthal DL, Paddock D, Storey K, Lea K (2000) Complex regulation of the yeast heat shock transcription factor. Mol Biol Cell 11:1739–1751

Chen Y, Brandizzi F (2013) IRE1: ER stress sensor and cell fate executor. Trends Cell Biol 23:547–555

Cheney CM, Shearn A (1983) Developmental regulation of Drosophila imaginal disc proteins: synthesis of a heat shock protein under non-heat-shock conditions. Dev Biol 95:325–330

Chu B, Soncin F, Price BD, Stevenson MA, Calderwood SK (1996) Sequential phosphorylation by mitogen-activated kinase and glycogen synthase kinase 3 represses transcriptional activation by heat shock factor-1. J Biol Chem 271:30847–30857

Clos J, Rabindran S, Wisniewski J, Wu C (1993) Induction temperature of human heat shock factor is reprogrammed in a Drosophila cell environment. Nature 364:252–255

Cotto JJ, Morimoto RI (1999) Stress-induced activation of the heat-shock response: cell and molecular biology of heat-shock factors. Biochem Soc Symp 64:105–118

Cuesta R, Laroia G, Schneider RJ (2000) Chaperone hsp27 inhibits translation during heat shock by binding eIF4G and facilitating dissociation of cap-initiation complexes. Genes Dev 14:1460–1470

de la Cadena SG, Hernández-Fonseca K, Camacho-Arroyo I, Massieu L (2013) Glucose deprivation induces reticulum stress by the PERK pathway and caspase-7- and calpain-mediated caspase-12 activation. Apoptosis 19:414–427

Ding XZ, Tsocos GC, Kiang JG (1997) Heat shock factor-1 in heat shock factor-1 gene-transfected human epidermoid A431 cells requires phosphorylation before inducing heat shock protein-70 production. J Clin Invest 99:136–143

Dubois MF, Marshall NF, Nguyen VT, Dacmus GK, Bonnet F et al (1999) Heat shock of HeLa cells inactivates a nuclear protein phosphatase specific for dephosphorylation of the C-terminal domain of RNA polymerase II. Nucleic Acids Res 27:1338–1344

Duncan RF, Cavener DR, Qu S (1995) Heat shock effects on phosphorylation of protein synthesis initiation factor proteins eIF4E and eIF2-alpha in Drosophila. Biochemistry 34:2985–2997

Foti DM, Welihinda A, Kaufman RJ, Lee AS (1999) Conservation and divergence of the yeast and mammalian unfolded protein response. J Biol Chem 274:30402–30409

Fujimoto M, Hayashida N, Katoh T, Oshima K, Shinkawa T et al (2010) A novel mouse HSF3 has the potential to activate nonclassical heat-shock genes during heat shock. Mol Biol Cell 21:106–116

Gallie DR, Le H, Caldwell C, Tanduay RL, Hoang NX, Browning KS (1997) The phosphorylation state of translation initiation factors is regulated developmentally and following heat shock in wheat. J Biol Chem 272:1046–1053

Gallo GJ, Schuetz TJ, Kingston RE (1991) Regulation of heat shock factor in Schizosaccharomyces pombe more closely resembles regulation in mammals than in Saccharomyces cerevisiae. Mol Cell Biol 11:281–288

Georgel PT (2005) Chromatin potentiation of the hsp70 promoter is linked to GAGA-factor recruitment. Biochem Cell Biol 83:555–565

Gonsalves SE, Moses AM, Razak Z, Robert F, Westwood JT (2011) Whole-genome analysis reveals that active heat shock factor binding sites are mostly associated with non-heat shock genes in Drosophila melanogaster. PLoS One 6:e15934

Guertin MJ, Lis JT (2010) Chromatin landscape dictates HSF binding to target DNA elements. PLoS Genet 6:e1001114

Guertin MJ, Lis JT (2013) Mechanisms by which transcription factors gain access to target sequence elements in chromatin. Curr Opin Genet Dev 23:116–123

Guertin MJ, Petesch SJ, Zobeck KL, Min IM, Lis JT (2010) Drosophila heat shock system as a general model to investigate transcriptional regulation. Cold Spring Harb Symp Quant Biol 75:1–9

Guettouche T, Boellmann F, Lane WS, Voellmy R (2005) Analysis of phosphorylation of human heat shock factor 1 in cells experiencing a stress. BMC Biochem 6:4

Hahn JS, Thiele DJ (2004) Activation of the Saccharomyces cerevisiae heat shock transcription factor under glucose starvation conditions by Snf1 protein kinase. J Biol Chem 279:5169–5176

Harding HP, Novoa I, Zhang Y, Zeng H, Wek R et al (2000) Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell 6:1099–1108

Hart C, Zhao K, Laemmli U (1997) The scs' boundary element: characterization of boundary element-associated factors. Mol Cell Biol 17:999–1009

Hashikawa N, Mizukami Y, Imazu H, Sakurai H (2006) Mutated yeast heat shock transcription factor activates transcription independently of hyperphosphorylation. J Biol Chem 281:3936–3942

He B, Meng Y, Mivechi NF (1998) Glycogen synthase kinase 3β and extracellular signal-regulated kinase inactivate heat shock transcription factor 1 by facilitating the disappearance of transcriptionally active granules after heat shock. Mol Cell Biol 18:6624–6633

Hernández G, Vázquez-Pianzola P, Sierra JM, Rivera-Pomar R (2004) Internal ribosome entry site drives cap-independent translation of reaper and heat shock protein 70 mRNAs in Drosophila embryos. RNA 10:1783–1797

Holmberg CI, Hietakangas V, Mikhailov A, Rantanen JO, Kallio M et al (2001) Phosphorylation of serine 230 promotes inducible transcriptional activity of heat shock factor 1. EMBO J 20:3800–3810

Huang J, Van der Ploeg LHT (1991) Maturation of polycistronic pre-mRNA in Trypanosoma brucei: analysis of trans-splicing and Poly(A) addition at nascent RNA transcripts from the *hsp70* locus. Mol Cell Biol 11:3180–3190

Jacoby DB, Wensink PC (1994) Yolk protein factor 1 is a Drosophila homolog of Ku, the DNA-binding subunit of a DNA-dependent protein kinase from humans. J Biol Chem 269: 11484–11491

Karpov VL, Preobrazhenskaya OV, Mirzabekov AD (1984) Chromatin structure of *hsp*70 genes, activated by heat shock: selective removal of histones from the coding region and their absence from the 5′ region. Cell 36:423–431

Kiang JG, Gist ID, Tsokos GC (1998) Cytoprotection and regulation of heat shock proteins induced by heat shock in human breast cancer T47-D cells: role of (Ca2+)i and protein kinases. FASEB J 12:1571–1579

Kim D, Ouyang H, Yang SH, Nussenzweig A, Burgman P, Li GC (1995) A constitutive heat shock element-binding factor is immunologically identical to the Ku autoantigen. J Biol Chem 270:15277–15284

Kinoshita K, Shinka T, Sato Y, Kurahashi H, Kowa H et al (2006) Expression analysis of a mouse orthologue of HSFY, a candidate for the azoospermic factor on the human Y chromosome. J Med Invest 53:117–122

Korochkin LI, Aleksandrova MA, Bashkirov VN, Trukhacheva AA, Dzitoyeva SG et al (2002) Xenografts of embryonic nerve tissue from Drosophila neuromutants stimulate development of neural homografts in rat brain and block glial scar formation. Tsitologiia 44: 1181–1185

Kruger C, Benecke BJ (1981) In vitro translation of Drosophila heat-shock and non-heat-shock mRNAs in heterologous and homologous cell-free systems. Cell 23:595–603

Lebedeva LA, Nabirochkina EN, Kurshakova MM, Robert F, Krasnov AN et al (2005) Occupancy of the Drosophila hsp70 promoter by a subset of basal transcription factors diminishes upon transcriptional activation. Proc Natl Acad Sci U S A 102:18087–18092

Lee AS (2001) The glucose-regulated proteins: stress induction and clinical applications. Trends Biochem Sci 26:504–510

Lee H, Kraus K, Wolfner M, Lis J (1992) DNA sequence requirements for generating paused polymerase at the start of hsp70. Genes Dev 6:284–285

Lee C, Li X, Hechmer A, Eisen M, Biggin MD et al (2008a) NELF and GAGA factor are linked to promoter-proximal pausing at many genes in Drosophila. Mol Cell Biol 28: 3290–3300

Lee K, Park JY, Yoo W, Gwag T, Lee JW, Byun MW, Choi I (2008b) Overcoming muscle atrophy in a hibernating mammal despite prolonged disuse in dormancy: proteomic and molecular assessment. J Cell Biochem 104:642–656

Lerman DN, Feder ME (2005) Naturally occurring transposable elements disrupt hsp70 promoter function in *Drosophila melanogaster*. Mol Biol Evol 22:776–783

Li WW, Hsiung Y, Zhou Y, Roy B, Lee AS (1997) Induction of the mammalian GRP78/BiP gene by Ca2+ depletion and formation of aberrant proteins: activation of the conserved stress-inducible grp core promoter element by the human nuclear factor YY1. Mol Cell Biol 17:54–60

Lis JT (2007) Imaging Drosophila gene activation and polymerase pausing in vivo. Nature 450:198–202

Loones MT, Rallu M, Mezger V, Morange M (1997) HSP gene expression and HSF2 in mouse development. Cell Mol Life Sci 53:179–190

Marchler G, Wu C (2001) Modulation of Drosophila heat shock transcription factor activity by the molecular chaperone DROJ1. EMBO J 20:499–509

Menon V, Thomason DB (1995) Head-down tilt increases rat cardiac muscle eIF2α phosphorylation. Am J Physiol 269:802–804

Morgan WD, Williams GT, Morimoto RI, Greene J, Kingston RE, Tjian R (1987) Two transcriptional activators, CCAAT-box-binding transcription factor and heat shock factor, interact with a human *HSP70* gene promoter. Mol Cell Biol 7:1129–1138

Mori K, Kawahara T, Yoshida H, Yanagi H, Yura T (1996) Signaling from endoplasmic reticulum to nucleus: transcription factor with a basic-leucine zipper motif is required for the unfolded protein-response pathway. Genes Cells 1:803–817

Mori K, Ogawa N, Kawahara T, Yanagi H, Yura T (1998) Palindrome with spacer of one nucleotide is characteristic of the cis-acting unfolded protein response element in saccharomyces cerevisiae. J Biol Chem 273:9912–9920

Morimoto RI (1998) Regulation of the heat shock transcription response: cross talk between a family of HSFs, molecular chaperones, and negative regulators. Genes Dev 12:3788–3796

Morita MT, Tanaka Y, Kodama TS, Kyogoku Y, Yanagi H, Yura T (1999) Translational induction of heat shock transcription factor σ-32: evidence for a built-in RNA thermosensor. Genes Dev 13:655–665

Nikolova-Karakashian MN, Rozenova KA (2010) Ceramide in stress response. Adv Exp Med Biol 688:86–108

Nussenzweig A, Chen C, da Costa Soares V, Sanchez M, Sokol K et al (1996) Requirement for Ku80 in growth and immunoglobulin V(D)J recombination. Nature 382:551–555

Omelina ES, Baricheva EM, Oshchepkov DY, Merkulova TI (2011) Analysis and recognition of the GAGA transcription factor binding sites in Drosophila genes. Comput Biol Chem 35:363–370

Orosz A, Wisniewski J, Wu C (1996) Regulation of Drosophila heat shock factor trimerisation: global sequence requirements and independence of nuclear localization. Mol Cell Biol 16:7018–7030

Ostling P, Björk JK, Roos-Mattjus P, Mezger V, Sistonen L (2007) Heat shock factor 2 (HSF2) contributes to inducible expression of hsp genes through interplay with HSF1. J Biol Chem 282:7077–7086

Papaconstantinou M, Wu Y, Pretorius HN, Singh N, Gianfelice G et al (2005) Menin is a regulator of the stress response in Drosophila melanogaster. Mol Cell Biol 25:9960–9972

Park JM, Werner J, Kim JM, Lis JT, Kim YJ (2001) Mediator, not holoenzyme, is directly recruited to the heat shock promoter by HSF upon heat shock. Mol Cell 8:9–19

Peng W, Zhang Y, Zheng M, Cheng H, Zhu W et al (2010) Cardioprotection by CaMKII-deltaB is mediated by phosphorylation of heat shock factor 1 and subsequent expression of inducible heat shock protein 70. Circ Res 106:102–110

Petesch S, Lis J (2012) Activator-induced spread of poly(ADP-ribose) polymerase promotes nucleosome loss at Hsp70. Mol Cell 45:64–74

Petesch SJ, Lis JT (2008) Rapid, transcription-independent loss of nucleosomes over a large chromatin domain at Hsp70 loci. Cell 134:74–84

Pirkkala L, Alastalo TP, Zuo X, Benjamin IJ, Sistonen L (2000) Disruption of heat shock factor 1 reveals an essential role in the ubiquitin proteolytic pathway. Mol Cell Biol 20:2670–2675

Place RF, Noonan EJ (2014) Non-coding RNAs turn up the heat: an emerging layer of novel regulators in the mammalian heat shock response. Cell Stress Chaperones 19:159–172

Rabindran SK, Haroun RI, Clos J, Wisniewski J, Wu C (1993) Regulation of heat shock factor trimer formation: role of a conserved leucine zipper. Science 259:230–234

Roy B, Lee AS (1999) The mammalian endoplasmic reticulum stress response element consists of an evolutionarily conserved tripartite structure and interacts with a novel stress-inducible complex. Nucleic Acids Res 27:1437–1443

Roy B, Li WW, Lee AS (1996) Calcium-sensitive transcriptional activation of the proximal CCAAT regulatory element of the grp78/BiP promoter by the human nuclear factor CBF/NF-Y. J Biol Chem 271:28995–29002

Rubtsova MP, Sizova DV, Dmitriev SE, Ivanov DS, Prassolov VS, Shatsky IN (2003) Distinctive properties of the 5′-untranslated region of human hsp70 mRNA. J Biol Chem 278: 22350–22356

Segal G, Ron EZ (1998) Regulation of heat-shock response in bacteria. Ann N Y Acad Sci 851:147–151

Shalgi R, Hurt JA, Krykbaeva I, Taipale M, Lindquist S, Burge CB (2013) Widespread regulation of translation by elongation pausing in heat shock. Mol Cell 49:439–452

Shamovsky I, Nudler E (2009) Isolation and characterization of the heat shock RNA 1. Methods Mol Biol 540:265–279

Shamovsky I, Ivannikov M, Kandel ES, Gershon D, Nudler E (2006) RNA-mediated response to heat shock in mammalian cells. Nature 440:556–560

Sheikh MS, Fornace AJ (1999) Regulation of translation following stress. Oncogene 18:6421–6428

Shi Y, Kroeger PE, Morimoto R (1995) The carboxyl-terminal transcription domain of heat shock factor 1 is negatively regulated and stress responsive. Mol Cell Biol 15:4309–4318

Shi Y, Mosser DD, Morimoto RI (1998) Molecular chaperones as HSF1-specific transcriptional repressors. Genes Dev 12:654–656

Shinka T, Sato Y, Chen G, Naroda T, Kinoshita K et al (2004) Molecular characterization of heat shock-like factor encoded on the human Y chromosome, and implications for male infertility. Biol Reprod 71:297–306

Shopland LS, Hirayoshi K, Fernandes M, Lis JT (1995) HSF access to heat shock elements *in vivo* depends critically on promoter architecture defined by GAGA-factor, TFIID, and RNA-polymerase II binding sites. Genes Dev 9:2756–2769

Simioni MB, De Thonel A, Hammann A, Joly AL, Bossis G et al (2009) Heat shock protein 27 is involved in SUMO-2/3 modification of heat shock factor 1 and thereby modulates the transcription factor activity. Oncogene 28:3332–3344

Singh IS, He JR, Calderwood S, Hasday JD (2002) A high affinity HSF-1 binding site in the 5′-untranslated region of the murine tumor necrosis factor-alpha gene is a transcriptional repressor. J Biol Chem 277:4981–4988

Solomon JM, Rossi JM, Golic K, McGarry T, Lindquist S (1991) Changes in Hsp70 alter thermotolerance and heat-shock regulation in Drosophila. New Biol 3:1106–1120

Sørensen JG, Nielsen MM, Kruhøffer M, Justesen J, Loeschcke V (2005) Full genome gene expression analysis of the heat stress response in *Drosophila melanogaster*. Cell Stress Chaperones 10:312–328

Stephanou A, Isenberg DA, Nakajima K, Latchman DS (1999) Signal transducer and activator of transcription-1 and heat shock factor-1 interact and activate the transcription of the HSP70 and HSP90β promoters. J Biol Chem 274:1723–1728

Tanabe M, Sasai N, Nagata K, Liu XD, Liu PC et al (1999) The mammalian HSF4 gene generates both an activator and a repressor of heat shock genes by alternative splicing. J Biol Chem 274:27845–27856

Tang D, Xie Y, Zhao M, Stevenson MA, Calderwood SK (2001) Repression of the HSP70B promoter by NFIL6, Ku70, and MAPK involves three complementary mechanisms. Biochem Biophys Res Commun 280:280–285

Thomas SR, Lengyel JA (1986) Ecdysteroid-regulated heat-shock gene expression during Drosophila melanogaster development. Dev Biol 115:434–438

Tian S, Haney RA, Feder ME (2010) Phylogeny disambiguates the evolution of heat-shock cis-regulatory elements in Drosophila. PLoS One 5:e10669

Tsukiyama T, Becker PB, Wu C (1994) ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. Nature 367:525–532

Turturici G, Geraci F, Candela ME, Cossu G, Giudice G, Sconzo G (2009) Hsp70 is required for optimal cell proliferation in mouse A6 mesoangioblast stem cells. Biochem J 421: 193–200

Venetianer A, Marie-Francoise D, Nguyen VT, Bellier S, Seo SJ, Bensaud O (1995) Phosphorylation state of the RNA polymerase II C-terminal domain (CTD) in heat shocked cells. Possible involvement of the stress-activated mitogen-activated protein (MAP) kinases. Eur J Biochem 233:83–92

Vera M, Pani B, Griffiths LA, Muchardt C, Abbott CM, Singer RH, Nudler E (2014) The translation elongation factor eEF1A1 couples transcription to translation during heat shock response. Elife 3:e03164

Vogel JL, Parsell DA, Lindquist S (1995) Heat-shock proteins Hsp104 and Hsp70 reactivate mRNA splicing after heat inactivation. Curr Biol 5:306–317

Vries RG, Flynn A, Patel JC, Wang X, Denton RM, Proud CG (1997) Heat shock increases the association of binding protein-1 with initiation factor 4E. J Biol Chem 272:32779–32784

Wang G, Ying Z, Jin X, Tu N, Zhang Y et al (2004a) Essential requirement for both hsf1 and hsf2 transcriptional activity in spermatogenesis and male fertility. Genesis 38:66–80

Wang X, Grammatikakis N, Siganou A, Stevenson MA, Calderwood SK (2004) Interactions between extracellular signal-regulated protein kinase 1, 14-3-3epsilon, and heat shock factor 1 during stress. J Biol Chem 279:49460–4946

Wells GB, Dickson RC, Lester RL (1998) Heat-induced elevation of ceramide in Saccharomyces cerevisiae via de novo synthesis. J Biol Chem 273:7235–7243

Westerheide SD, Anckar J, Stevens SM, Lea Sistonen L, Morimoto RI (2009) Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. Science 323:1063–1066

Westwood JT, Wu C (1993) Activation of Drosophila heat shock factor: conformational change associated with a monomer-to-trimer transition. Mol Cell Biol 13:3481–3486

Westwood JT, Clos J, Wu C (1991) Stress-induced oligomerization and chromosomal relocalization of heat-shock factor. Nature 353:822–827

Wilkins RC, Lis JT (1997) Dynamics of potentiation and activation: GAGA factor and its role in heat shock gene regulation. Nucleic Acids Res 25:3963–3968

Wu C (1995) Heat shock transcription factors: structure and regulation. Annu Rev Cell Dev Biol 11:441–469

Wu CH, Yamaguchi Y, Benjamin LR, Horvat-Gordon M, Washinsky J et al (2003) NELF and DSIF cause promoter proximal pausing on the hsp70 promoter in Drosophila. Genes Dev 17:1402–1414

Yamamoto A, Mizukami Y, Sakurai H (2005) Identification of a novel class of target genes and a novel type of binding sequence of heat shock transcription factor in Saccharomyces cerevisiae. J Biol Chem 280:11911–11919

Yang SH, Nussenzweig A, Li L, Kim D, Ouyang H et al (1996) Modulation of thermal induction of hsp70 expression by Ku autoantigen or its individual subunits. Mol Cell Biol 16:3799–3806

Yao J, Munson KM, Webb WW, Lis JT (2006) Dynamics of heat shock factor association with native gene loci in living cells. Nature 442:1050–1053

Yueh A, Schneider RJ (2000) Translation by ribosome shunting on adenovirus and *hsp70* mRNAs facilitated by complementarity to 18S rRNA. Genes Dev 14:414–421

Zhang M, Buckley D, Lavoi KP, Buckley AR, Blake MJ (1998) Heat-induced proteolysis of HSF causes premature deactivation of heat shock response in Nb2 lymphoma cells. Cell Stress Chaperones 3:57–66

Zhang M, Blake MJ, Gout PW, Buckley DJ, Buckley AR (1999) Proteolysis of heat shock transcription factor is associated with apoptosis in rat Nb2 lymphoma cells. Cell Growth Differ 10:759–767

Zimarino V, Tsai C, Wu C (1990) Complex modes of heat shock factor activation. Mol Cell Biol 10:752–759

Zou J, Guo Y, Guettouche T, Smith DF, 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