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REVIEWS

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Regulation of Heat Shock Gene Expression in Response to Stress

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Abstract—Heat shock (HS) genes, or stress genes, code for a number of proteins that collectively form the most ancient and universal stress defense system. The system determines the cell capability of adaptation to various adverse factors and performs a variety of auxiliary functions in normal physiological conditions. Common stress factors, such as higher temperatures, hypoxia, heavy metals, and others, suppress transcription and translation for the majority of genes, while HS genes are upregulated. Transcription of HS genes is controlled by transcription factors of the HS factor (HSF) family. Certain HSFs are activated on exposure to higher temperatures or other adverse factors to ensure stress-induced HS gene expression, while other HSFs are specifically activated at particular developmental stages. The regulation of the main mammalian stress-inducible factor HSF1 and *Drosophila melanogaster* HSF includes many components, such as a variety of early warning signals indicative of abnormal cell activity (e.g., increases in intracellular ceramide, cytosolic calcium ions, or partly denatured proteins); protein kinases, which phosphorylate HSFs at various Ser residues; acetyltransferases; and regulatory proteins, such as SUMO and HSBP1. Transcription factors other than HSFs are also involved in activating HS gene transcription; the set includes *D. melanogaster* GAF, mammalian Sp1 and NF-Y, and other factors. Transcription of several stress genes coding for molecular chaperones of the glucose-regulated protein (GRP) family is predominantly regulated by another stress-detecting system, which is known as the unfolded protein response (UPR) system and is activated in response to massive protein misfolding in the endoplasmic reticulum and mitochondrial matrix. A translational fine tuning of HS protein expression occurs via changing the phosphorylation status of several proteins involved in translation initiation. In addition, specific signal sequences in the 5'-UTRs of some HS protein mRNAs ensure their preferential translation in stress.

Keywords: heat shock, heat shock proteins, transcription regulation

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INTRODUCTION

Various stress factors (higher temperatures, hypoxia, oxidative stress, virus infection, etc.) activate a group of genes known as the heat shock (HS) genes, or stress genes. Their protein products (HS proteins (HSPs)) perform multiple protective functions in the cell, acting mostly to prevent aggregation of partly denatured proteins, to allow their refolding, and to stimulate degradation of irreversibly damaged proteins. The proteins that nonspecifically interact with various partly denatured proteins to prevent their aggregation and to facilitate the restoration of their native structures are known as the molecular chaperones (from French chaperone, a companion). HSPs are classed into several families by molecular weight: small HSPs (sHSPs) with molecular weights of 10–30 kDa, Hsp40 (40 kDa), Hsp60 (chaperonines), Hsp70 (68–78 kDa), Hsp90 (82–96 kDa), Hsp100 (a heterogeneous group of proteins with molecular weights of approximately 78–104 kDa), and Hsp110 (a group of proteins with molecular weights varying within a broad range of 80–170 kDa) [1–3].

In addition to the classification by molecular weight of their protein products, HS genes are divided into two large groups by expression pattern, inducible or constitutive. Genes of the former group are normally expressed to extremely low levels (e.g., reverse transcription–polymerase chain reaction is necessary to detect the *hsp70* mRNA in *Drosophila melanogaster*), while the intensity of *hsp70* transcription increases in stress by a factor of several hundred or several thousand relative to the baseline. Constitutive genes are expressed to relatively high levels even at normal temperatures, and their transcription in stress increases only several fold, that is, not as dramatically as that of the inducible genes [4, 5]. Glucose-regulated protein (GRP) genes are sometimes isolated in a separate group; their expression increases in response to glucose deficiency, N-glycosylation inhibitors, and other agents that dis-

tort protein folding in the endoplasmic reticulum and mitochondria [6].

Activation of the HS genes in stress occurs at various steps of expression, predominantly at the transcriptional and translational levels. In prokaryotes, RNA polymerase σ factors regulate expression of various genes by recognizing certain nucleotide sequences and targeting RNA polymerase to specific promoters. The σ32 factor, which is encoded by *rpoH*, ensures transcription from HS gene promoters in *Escherichia coli*. The HS gene expression level strongly positively correlates with the intracellular σ32 level. In normal conditions, transcription of *groES* and *groEL*, which code for Hsp60-family chaperonines, is maintained at a low level by the primary vegetative factor σ70. The RNA polymerase complex with σ70 is destabilized in HS, and σ70 is replaced with σ32. The σ32 is not involved in active transcription in the absence of stress because proteases Hf1B and ClpP cleave it at the C end so that its half-life is ~30 s in normal conditions [7]. Stress dramatically increases the cell concentration of damaged proteins, which also act as protease targets. The proteases consequently switch to new substrates, and the σ32 half-life considerably increases. The level of σ32 mRNA translation additionally increases in stress. A unique secondary structure that forms at the 5' end of the σ32 mRNA and involves the 5'-untranslated region (5'-UTR) attenuates translation. The 5' secondary structure melts at a higher temperature, thus abolishing the translation block and increasing the efficiency of σ32 mRNA translation [8]. Thus, σ32 activity is regulated at the translational and posttranslational levels.

In eukaryotes (Metazoa in particular), both transcriptional and translational regulation is known for HS genes. Transcriptional activation of HS genes is visually detectable in various *Drosophila* species by light microscopy of polytene chromosomes from the larval salivary glands. When the temperature becomes 8–10°C higher than the developmental optimum (~25°C in the case of *D. melanogaster*), decondensed chromatin regions known as the puffs form in several chromosome loci, where HS genes occur. The process is very fast, taking approximately 1 min [9, 10]. This rapid activation of the HS response is essential for the cell to have time enough to adapt to stress factors until irreversible damage occurs. The processes involved in regulating expression of the HS genes are hereafter considered predominantly for Metazoa.

REGULATORY *cis* ELEMENTS IN PROMOTERS OF HS GENES

Conserved sequences known as the heat shock elements (HSEs) are found upstream of the TATA box at the 5' ends of the HS genes in all eukaryotes examined. The optimal context is 5'-NAGAANNTTCNN-GAANN-3', where N is any nucleotide [11]. A GGA trinucleotide unit (or its complementary unit TTC) plays a main functional role in the sequence. The guanine in the first position is the most important, while substitutions for the adenines in the second and especially the third position do not as greatly affect the HSE function. In addition to typical HSEs, there are HSEs that contain two inverted units separated from a third unit by 5 bp (nTTCnnGAAn(5 bp)nGAAn) or direct repeats separated from each other by 5 bp (nGAAn(5 bp)nGAAn(5 bp)nGAAn) [12, 13]. One HSE copy is already functional. Yet multiple HSE copies occur in the majority of the known HS gene promoters. For instance, four HSEs are located at various distances of each other in the regulatory region of *D. melanogaster hsp70* [14, 15]. Transcription is more intense in the presence of multiple HSE copies. When an HSE is experimentally inserted in a promoter, the respective gene becomes inducible in hyperthermia, like the HS genes [16].

High conservation of HSEs and the HS response in eukaryotes is evident from many experiments wherein the *D. melanogaster hsp70* promoter efficiently functioned in cells of various, including phylogenetically distant, organisms. For instance, reporter constructs controlled by the *hsp70* regulatory region of *D. melanogaster* (family Drosophilidae, order Diptera), were efficiently transcribed in cells of the mosquito *Aedes aegypti* (Diptera), transgenic silkworm *Bombyx mori* strains (Hymenoptera), and sea urchin embryos [17–19]. Constructs containing *D. melanogaster hsp70* along with its 5'-terminal regulatory region were efficiently transcribed in clawed frog *Xenopus laevis* oocytes, rat fibroblasts, and monkey COS cells [20–23]. A distinct stress-inducible expression pattern was observed for all constructs in alien host cells, testifying again to high conservation of stress response mechanisms among different organisms. On the other hand, a far lower efficiency of the *D. melanogaster hsp70* promoter compared with its host counterpart was observed in some cases, including cells of other Diptera species. For instance, expression of the chloramphenicol acetyltransferase gene under the control of the *D. melanogaster hsp70* promoter in transgenic *Lucilia cuprina* flies was 10- to 100-fold lower than in *Drosophila* cells and, most interestingly, showed no induction in stress [24]. When transgenic *Ceratitis capitata* flies were engineered to carry *lacZ* under the control of the *D. melanogaster* or the cognate *C. capitata hsp70* promoter, the heterologous promoter was five- to sixfold less efficient than the cognate one [25]. A transgene construct under the control of the *hsp70* promotor of *Stratiomys singularior* (Diptera, Stratiomyidae) was not transcribed, nor did it cause chromosome puffing in the sites of its integration in the *D. melanogaster* genome [26]. All these species belong to different families of the same order Diptera and are therefore relatively close phylogenetically. The results indicate that certain specifics of the HS response regulation arose in some organisms in the course of their evolution.

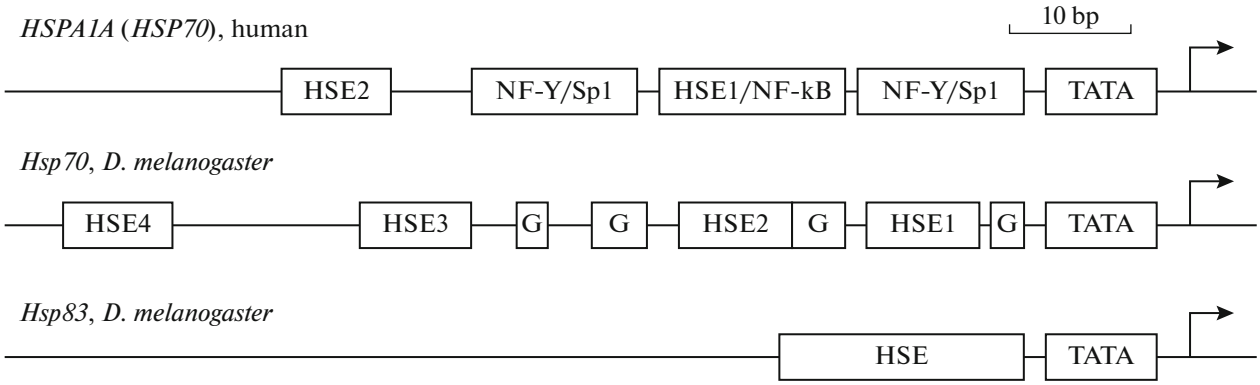

Fig. 1. Positions of regulatory elements in the promoters of several HS genes. The transcription start is shown with a broken arrow; G is a GAGA site. The box length is proportional to the effect that the corresponding site exerts on transcription.

In addition to the HSE, the TATA box and certain other regulatory elements are necessary for the normal function of the promoter in the majority of inducible HS genes (Fig. 1). For instance, CCAAT and Sp1 binding sites are found in the mammalian *HSP70* genes [27, 28]. Binding sites for NF-κB and STAT-3 occur in the *HSPA1A* and *HSPA1B* promoters [29]. A set of binding sites for the so-called GAGA factor, which is a product of *trithorax-like*, is required for stress-induced transcription from the promoters of *hsp70* sHsp genes in *Drosophila*. The GAGA factor is necessary for RNA polymerase II positioning and histone modification in the transcription region [30–33]. Thus, many transcription factors are involved in stress-induced transcriptional activation, and several pathways are possible for activation of the HS genes in response to physical, chemical, and biochemical signals varying in nature (HS, protein-denaturing agents, cytokines, etc.).

SYSTEM THAT REGULATES ACTIVITY OF THE MAIN TRANSCRIPTION FACTOR MEDIATING TRANSCRIPTION OF THE HS GENES (HSF)

A family of transcription factors was identified to specifically bind to the HSE sequence and to regulate activity of the HS genes in normal conditions, in HS, and at certain stages of embryo development and tissue differentiation. The proteins are termed the HS transcription factors (HSFs, or HSTFs). At least five members of the HSF family are currently known for vertebrates: HSF1, HSF2, HSF3, HSF4, and HSFY [34–37]. HSF1 is activated in HS and several other stress types and ensures inducible transcription of the HS genes. HSF2 is not activated in response to stress exposures, but ensures constitutive *hsp* expression and HSP synthesis during embryo development [35, 38]. HSF3, which was initially identified in birds, is similar to HSF1 in being temperature inducible, but is activated at higher temperatures. A similar gene was found in the mouse genome and designated as *HSF3* [39]. The transcription factor encoded by the gene is incapable of inducing *hsp70,* but is responsible for activation of several nonclassical HS genes. *HSF4* was cloned from the mouse, rat, and human genomes [34, 35]. The fifth mammalian HSF family member HSFY is encoded by a Y-chromosomal gene. There is evidence that a deletion of the gene leads to azoospermia [40]. Only one gene of the *hsf* family was identified in *Drosophila* and was shown to have four splicing variants [41]. A plant system regulating the HS response is far more complex than the animal system; i.e., there are 21 HSF-coding genes in the *Arabidopsis thaliana* genome and 52 HSF-coding genes in the soybean genome [42].

HSFs from different organisms have up to 40% homology, and their secondary and tertiary structures are highly conserved [35]. The structure of mammalian HSF1 is considered below as an example. A DNA-binding domain, which recognizes the SHE sequence in the promoters of HS genes, is at the N end of the molecule and has a helix–turn–helix structure. Two domains, AD1 and AD2, at the C end (amino acid residues 395–503) modulate activity of the transcription protein complex. Region 137–407 includes elements that are responsible for regulating HSF1 activity in different conditions (normal temperatures and HS). Region 137–212 consists of three heptapeptide repeats, which form leucine zippers responsible for trimerization and are known as hydrophobic repeats A/B (HR-A/B). In addition to the three leucine zippers of region 137–212, a fourth one occurs in HSF1 region 378–407 (HR-C) and plays a role in the negative regulation of HSF1 activity via intramolecular interactions with the HR-A/B leucine zippers in the absence of stress. A deletion of this region leads to constitutive trimerization, and HSF1 assumes a DNA-binding conformation at normal temperatures [43–46]. Another negative regulatory element is in region 203–227 and inhibits activity of the transactivation domain at normal conditions [35, 44]. Region

300–310 is also important for the negative regulation of the HSF1 function, providing a target for constitutive phosphorylation.

The activation of HSF1 has steps that are common in different organisms. In general, the HS response system is highly evolutionarily conserved; e.g., *D. melanogaster* HSF is capable of inducing transcription of the HS genes in mammalian cells [47]. In normal conditions, *D. melanogaster* HSF and vertebrate HSF1 occur as monomers in complex with Hsp90 in the cell, like many other transcription factors whose activity is regulated with chaperones of the class [48–51]. The interaction with Hsp90 stabilizes the intramolecular interactions between HR-A/B and HR-C and prevents HSF1 trimerization. At higher temperatures, the complex of HSF with chaperones dissociates, and HSF1 monomers form a trimer via intermolecular interactions between the HR-A/B leucine zippers. Trimeric HSF1 binds to the HSE [41, 46]. HSF1 and HSF2 can form heterotrimers, which activate transcription in response to various stress factors and developmental signals [52]. At the next step, HSF1 is phosphorylated at multiple serine and threonine sites and becomes capable of activating transcription. In contrast to the human and *Drosophila* factors, *Saccharomyces cerevisiae* HSF lacks the HR-C leucine zipper and consequently occurs as a trimer in the cell at normal temperatures; its activation in HS is achieved only by phosphorylation and interactions with other transcriptional modulators [53]. *Drosophila* HSF and vertebrate HSF1 were assumed to act as temperature sensors; i.e., the conformational change resulting in trimerization is directly determined by changes in temperature. It was observed that *Drosophila* HSF overexpressed in HeLa cells is constitutively active [47]. Temperatures of 20–25°C are normal for *Drosophila* cells, while a temperature of 37°C is used to culture mammalian cells. The temperature causes stress in *Drosophila*, leading to HSF activation and HSP synthesis. On the other hand, the activation temperature of human HSF1 decreases by 10°C when the factor is overexpressed in *Drosophila* cells. It is clear that other cell systems are also involved in HSF activation in this case.

Trimerization of HSF1 is possible to induce at normal temperatures by introducing denatured proteins, for instance, bovine serum albumin, into the cell [54]. Another means to activate HSF1 is provided by proteasome inhibitors, which cause an accumulation and aggregation of polyubiquitinated proteins [55]. Geldanamycin, which blocks Hsp90 activity and causes dissociation of Hsp90 complexes with target proteins, also induces HSF1 trimerization and conversion to a DNA-binding form in the absence of HS. A current model assumes that HSF1 occurs in complex with the Hsp90 dimer, cochaperone p23, and immunophilin FKBP4 (the so-called temperature-sensitive multichaperone complex) in normal conditions. Hsp90 maintains HSF1 in an inactive conformation, which is due to the intramolecular hydrophobic interaction between the A/B and C leucine zippers. When denatured proteins accumulate in the cytoplasm, Hsp90 interacts with them, the interaction being mediated by Hsp70, another molecular chaperone. Because Hsp90 has higher affinity for these substrates than for HSF, the temperature-sensitive complex dissociates as a result [42]. Free HSF1 assumes the conformation that allows its trimerization and DNA binding.

In the recovery period after HS, HSPs regulate HSF activity via a common feedback mechanism. In *Drosophila*, Hsp70 binds with main transcriptionally active puffs in HS and colocalizes with HSF. Acting together with Hsp40, Hsp70 forms complexes with HSF both in vivo and in vitro; the complexes dissociate when ATP is added. The site involved in interactions with Hsp70 is in the region of the C-terminal domain of HSF. Overexpression of *Hsp70* under the control of a constitutive promoter suppresses HSF activity and decreases transcription from HSE-dependent promoters. HSF remains trimeric in this case, and its binding with DNA is not affected. Thus, Hsp70 and Hsp40 affect only transcriptional activity of HSF, apparently, by preventing HSF from being involved in transcription machinery. Then Hsp90 interacts with HSF to stimulate its monomerization and monomer refolding [37]; i.e., Hsp70 and Hsp90 prevent transcription of the HS genes and their own excess accumulation after HS.

Stress-induced phosphorylation follows trimerization at the next step of HSF activation. The process involves Ca^{2+}/calmodulin-dependent protein kinase (CaMKII), protein kinase C (PKC), DNA-dependent protein kinase (DNA-PK), and cAMP-dependent protein kinase A (PKA) according to current data. Activation of CaMKII in HS and hypoxia was shown to protect cardiomyocytes from apoptosis owing to HSF1 phosphorylation and Hsp70 induction [56]. CaMKII inhibitors, such as calphostin C, staurosporine, and the chelating agent BAPTA, reduce HSF1 activity and temperature-induced accumulation of the Hsp70 and Hsp27 mRNAs in human glioblastoma cells. PKC agonists, such as phorbol 12-myristate 13-acetate (PMA) and ionomycin, exert an opposite effect, which is accompanied by ^{32}P incorporation in HSF1 and HSF1 hyperactivation [57].

Protein kinases that stimulate HSF1 activity can be activated in HS as a result of increasing concentrations of ceramide and cAMP or a release of Ca^{2+} ions from the endoplasmic reticulum. Different protein kinases possibly phosphorylate HSF1 on exposure to different stress factors. In total, 12 serine phosphorylation sites were identified in HSF1, including positions 121, 230, 292, 303, 307, 314, 319, 326, 344, 363, 419, and 444. Phosphorylation at Ser326 plays a main role in HSF1 activation in HS [58]. In addition, there is evidence that threonine residues are phosphorylated in HSF1 in HeLa cells treated with sodium salicylate, while serine

residues are predominantly phosphorylated in cells exposed to HS [59]. The finding indicates that different HSF1 phosphorylation pathways most likely function on exposure to different stress factors and in different cell lines.

Various stress factors facilitate activation of several phospholipases (neutral and acid sphingomyelinases and phospholipases C, A_2, and D) in mammalian cells, leading to an increase in ceramide concentration as a result of sphingomyelin hydrolysis [60]. Ceramide concentration similarly increases in *S. cerevisiae* cells exposed to HS, but the increase is predominantly due to de novo ceramide synthesis with ceramide synthase and acetyl-CoA in contrast to what occurs in higher mammalian cells [61]. An elevated ceramide production may activate SAPK/JNK-family protein kinases and PKC. An increase in ceramide concentration alone in the absence of heat exposure is incapable of causing trimerization of HSF1 or stimulating its transcriptional activity, but leads to prolonged temperature-induced DNA-binding activity of HSF1 [60]. Thus, ceramide can be assumed to act as an early mediator in the HSF1 activation cascade and to activate HSF1 by influencing the HSF1 phosphorylation system without directly affecting HSF1 trimerization.

To summarize, there are two types of early mediators that the cell perceives as signals to activate the stress response systems. One is a higher concentration of denatured proteins, which lead to dissociation of the HSF complex with chaperones and cause HSF trimerization. The other includes an increase in concentrations of several low-molecular-weight compounds, such as ceramide and cAMP, and a release of calcium ions from the endoplasmic reticulum into the cytosol. These factors activate protein kinases, which then trigger various regulatory cascades [60]. Low-molecular-weight mediators, including cAMP, are essential components of many signaling pathways, for instance, those involved in transmission of certain hormonal signals, and their role in regulating the stress response is not surprising.

Phosphorylation of different serine residues can differently affect HSF1 activity. While phosphorylation at Ser326 activates HSF1, phosphorylation at Ser303 and Ser307 inactivates HSF1 and is necessary for a negative regulation of HS gene transcription. Phosphorylation at Ser303 and Ser307 occurs in the recovery period after stress and is driven by mitogen-activated/extracellular signal regulated (MAPK/ERK) protein kinases, p38/c-Jun N-terminal kinase (JNK), and glycogen synthase kinase 3β (GSK-3β). In addition, stress-inducible kinases p38 and JNK can rapidly inactivate HSF1 and thus prevent Hsp expression to facilitate apoptotic cell death in extremely severe stress, e.g., at temperatures lethal for the cell [62, 63].

A hierarchy is known for Ser303 and Ser307; i.e., Ser303 is not phosphorylated until Ser307 is. Recent data indicate that two mechanisms sustain the negative effect that Ser303 phosphorylation exerts on HSF1 activity. First, HSF1 phosphorylated at Ser303 binds with the protein 14-3-3ε, which facilitates the HSF1 transfer from the nucleus into the cytoplasm [64]. Second, Ser303 phosphorylation provides a signal for sumoylation of HSF1 at Lys298. SUMO1 and SUMO2/3 are involved in regulating HSF1 activity. The SUMO (small ubiquitin-related modifier) family includes a number of ubiquitin-like proteins, which bind with their substrates via isopeptide bonds and play a role in regulating activity of many transcription factors. SUMO1 binding inhibits both HSF1 and HSF2 transcriptional activities [63]. Like SUMO1, SUMO2/3 acts as a HSF1 inhibitor. It is of interest that Hsp27 is necessary for HSF1 modification with SUMO2/3 and is thereby capable of regulating expression of its own gene via negative feedback as it accumulates in the cell after HS and facilitates HSF1 inactivation [65].

Apart from the 14-3-3ε and SUMO proteins, HSF-binding protein 1 (HSBP1) specifically binds with the HSF1 trimer by interacting with the oligomerization domain (A/B leucine zippers) of HSF1 and thus inhibits HSF1 activity. HSBP1 overexpressed in cells decreases their resistance to HS and arsenite [66]. An unusual mechanism regulating the HS response was observed in Nb2 lymphoma cells; i.e., specific HSF proteolysis decreases the Hsp70 level. The protease potentially responsible for the process was characterized [67, 68].

Acetylation/deacetylation of the DNA-binding domain also regulates HSF1 activity in addition to phosphorylation. Acetylation at Lys80 abolishes DNA-binding activity of HSF1 and is a step in the negative regulation of the HS response. Acetyltransferase p300/cAMP response element-binding protein (CBP) is responsible for HSF1 acetylation in the recovery period after stress. Deacetylase SIRT1 (sirtuin) deacetylates HSF1 and thereby exerts an opposite effect, prolonging HSF1 activity and the HS response [69]. A general scheme of the HSF1 activity regulation in mammalian cells is shown in Fig. 2.

As mentioned above, HSF2, HSF3, HSF4, and HSFY were identified in vertebrates in addition to the main stress-regulated factor HSF1. HSF2 is incapable of inducing transcription of HS genes in stress. Its role is to ensure constitutive HSP expression and tissue-specific expression of certain HSPs act certain embryonic developmental stages (preimplantation development, cardiogenesis, and erythrocyte differentiation). Various embryonic tissues express two HSF2 isoforms, which result from alternative splicing and differ in transcriptional activity. Bird-specific HSF3 is activated in HS, but does not require phosphorylation in contrast to HSF1. HSF3 is constitutively expressed to a high level throughout the embryo development [37]. HSF4 is tissue-specifically expressed in heart, brain, and pancreatic cells and skeletal muscle fibers of

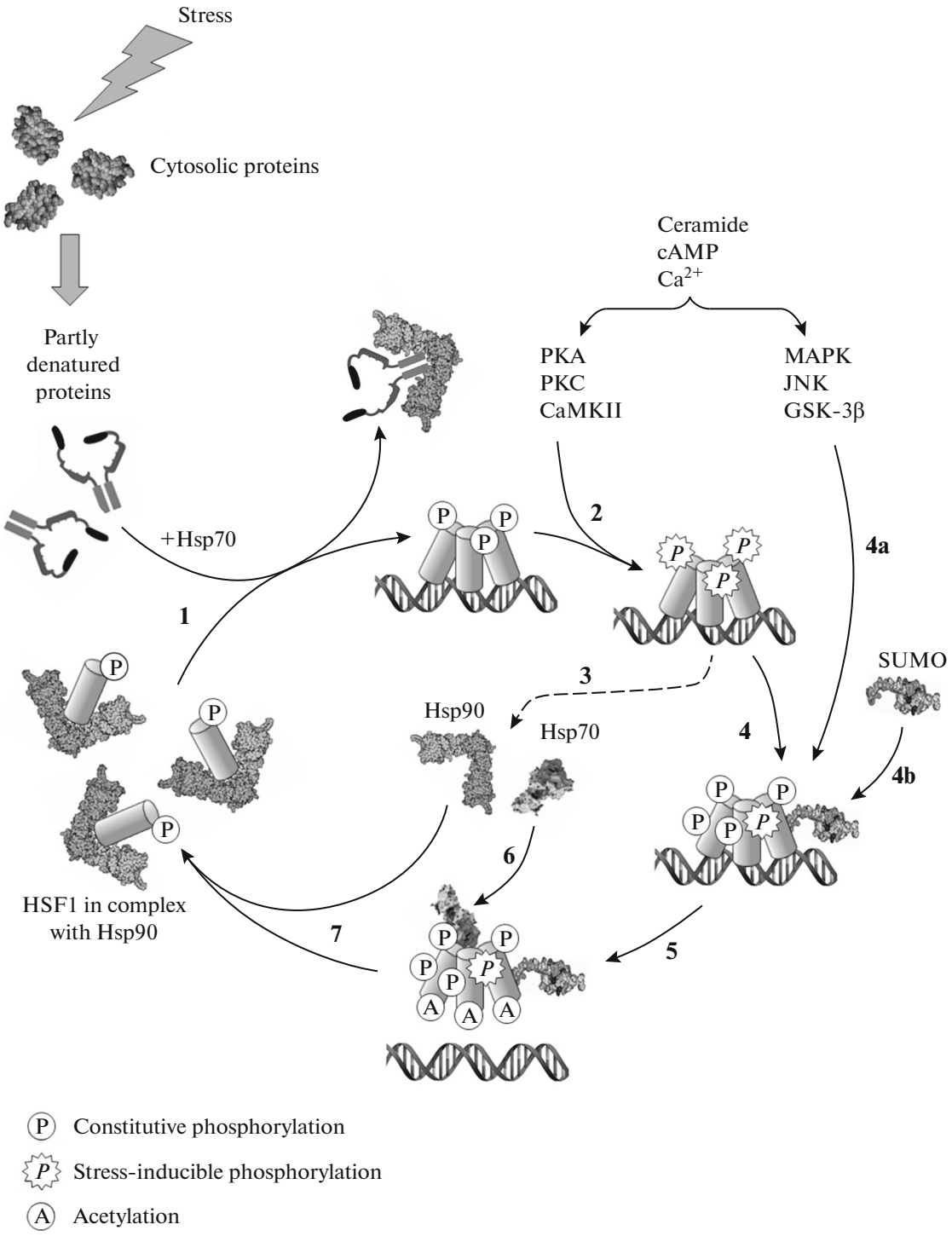

Fig. 2. Cycle of the regulation of HSF1 activity. In normal conditions, HSF1 occurs as a monomer in complex with Hsp70 and Hsp90. (1) A high concentration of denatured proteins in the cytosol results from HS to cause dissociation of the HSF1 complex with Hsp90 and HSF1 trimerization. Trimeric HSF1 interacts with DNA, but is incapable of activating transcription of the HS genes. (2) HSF1 is phosphorylated at multiple serine sites and acquires activity towards transcription machinery. Protein kinases involved in phosphorylating HSF are activated at higher concentrations of ceramide, cAMP, and calcium ions. (3) Transcription of the HS genes leads to an accumulation of Hsp70 and Hsp90 in the cell. (4) Inactivation of HSF1 involves its (4a) phosphorylation at Ser303 and Ser307 and (4b) sumoylation at Lys298. (5) The association of HSF1 with DNA is destabilized as HSF1 is acetylated by p300. (6) Hsp70 accumulating in the cell binds with HSF1 and abolishes its transcriptional activity. (7) Hsp90 facilitates HSF1 monomerization. Based on [37, 56, 63].

mammals. Alternative splicing of the HSF4 mRNA yields a transcriptional activator and a transcriptional repressor (isoforms a and b), which are involved in regulating transcription of the HS genes during embryo development. Isoform a acts as a transcriptional repressor; and isoform b, as a transcriptional activator. A deletion of the HR-C leucine zipper, which acts as a negative regulator, is a feature of HSF4 [70]. Data on the function of HSFY are scarce currently.

There is a hypothesis that a noncoding RNA known as HS RNA 1 (HSR1) is involved in activating HSF1 in mammals. Nudler and colleagues [71] assumed that HSR1 is highly structured and acts as a molecular thermometer by changing its tertiary structure at higher temperatures. In normal conditions, HSR1 occurs in complex with the translation elongation factor eEF1A. When the temperature increases and HSR1 changes its conformation, the HSR1/eEF1A complex interacts with HSF1 to facilitate its activation. It should be noted that the HSR1 nucleotide sequence was obtained as a result of reverse transcription of HSF1-associated RNPs isolated from HS-exposed HeLa cells by affinity chromatography. Its gene has not yet been found and localized in the human genome or another mammalian genome. Sequences homologous to HSR1 have not been found among eukaryotic sequences available from NCBI GenBank. There are data that HSR1 is an artifact resulting from bacterial contamination of the source material. This is evident from the fact that the HSR1 gene is highly homologous to a fragment of the Cl channel gene of several bacteria (*Comamonas*, *Burkholderia*, *Delftia*, and *Ralstonia*) [72, 73]. If so, the results [71] are probably artefactual and are due to aptamer activity of the highly structured RNA synthesized occasionally from a bacterial template.

TRANSCRIPTION FACTORS INVOLVED TOGETHER WITH HSFs IN TRANSCRIPTION OF HS GENES IN VARIOUS ORGANISMS

Eukaryotic transcription machinery is a highly complex multilevel system and requires the functions of many proteins, which ensure histone modification and subsequent chromatin decondensation in a transcribed region, destabilization of the DNA double helix, assembly of the initiation complex with RNA polymerase and general transcription factors (GRFs), the exact positioning of RNA polymerase relative to the transcription start site, and its modification on the transition to elongation. The HS genes are not an exclusion, and their transcription involves not only HSFs, but also many other proteins that are absolutely essential for a normal HS response.

Substantial progress has been achieved to date in studying the mechanisms of transcriptional activation of HS genes and *hsp70* in particular in *Drosophila*. It was found that RNA polymerase II molecules are constitutively associated with the promoter region (nucleotides –12...+65 relative to the transcription start) in the *hsp70* and small Hsp genes [74]. In addition, the 5' ends of the *hsp70* genes are free of histone H1 in both HS and control conditions; i.e., the promoters have a nucleosome-free open chromatin structure [75–77]. These features ensure a rapid response of transcription machinery to a higher temperature, and mRNA synthesis on the HS genes starts as early as 10–20 s after the start of exposure. The product of *trl* (*trithorax-like*) plays a crucial role in maintaining the open chromatin structure in the *hsp70* promoter regions and positioning RNA polymerase II in *Drosophila*. The protein is known as GAGA-binding factor (GAF) owing to its ability to bind to sequences consisting of several GA/CT repeats [78]. Several GAF binding sites occur within 150 bp upstream of the transcription start in the promoters of the *Drosophila hsp70* genes. Multiple GAGA sites located a certain distance apart in the direct (GAGA...) and inverse (TCTC...) orientation are strongly necessary for transcription of the *Drosophila hsp70* genes [79, 80]. GAF binding to the *hsp70* promoter provides a signal for histone H3 modification throughout the transcribed *hsp70* region leads to chromatin decondensation [76]. Efficient transcription elongation on *D. melanogaster hsp70* requires histone H3 be phosphorylated at S10 and methylated at K36 and histone H4 be acetylated [81–83]. There is evidence that the boundaries of DNA decondensation in the region of the *Drosophila hsp70* gene cluster are determined by the insulators scs (specialized chromatin structure) and scs' [30, 84], which are 1600-bp DNA regions that contain binding sites for BEAF2 and are capable of blocking the effect of an enhancer on a reporter gene (e.g., *white*). It is possible that BEAF2 is responsible for scs insulator activity [84].

Apart from chromatin modification, GAF is necessary for a positioning of RNA polymerase II in the transcription initiation region in complex with TFII-family proteins, in particular, the TATA box-binding protein (TBP). Other TFII-family factors are present in the promoter regions of the *hsp70* genes in normal conditions and dissociate in HS [85]. The binding of RNA polymerase II to the *Drosophila hsp70* promoters at a normal temperature is accompanied by synthesis of a short (20- to 40-nt) mRNA fragment, and then RNA polymerase passes into a transcriptionally paused state with the help of the negative elongation factor (NELF) and Spt4/5 (also known as the DRB sensitivity inducing factor (DSIF)). As active HSF interacts with the transcription complex in HS, RNA polymerase is hyperphosphorylated in the region of the large subunit C-terminal domain (CTD) by protein kinase P-TEFb and NELF dissociates [86, 87]. RNA polymerase thus becomes capable of continuing mRNA synthesis; i.e., transcription proceeds to the elongation stage (Fig. 3). *Drosophila* HSF does not directly interact with RNA polymerase II, and its effect is mediated by a mediator complex. HSF directly binds with a subunit of the mediator TRAP80 (also known as MED17) [88]. In

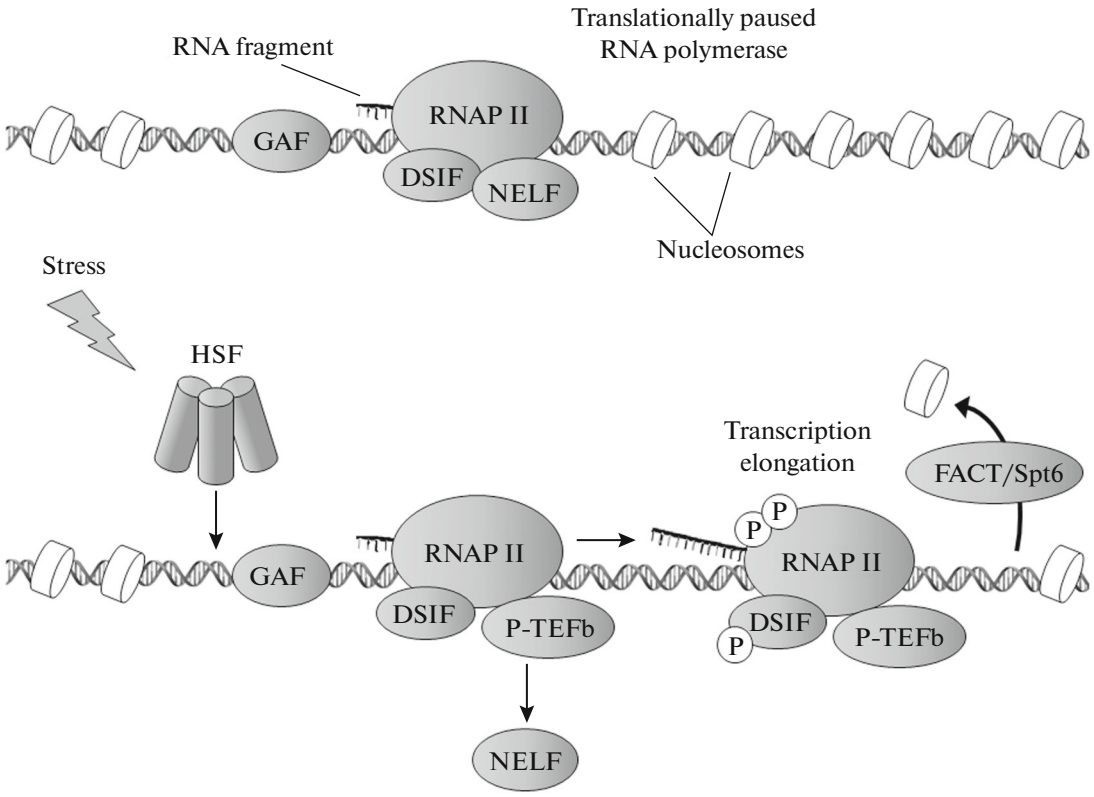

Fig. 3. Role of the GAGA-binding factor (GAF) in activating *hsp70* transcription in *D. melanogaster*. At the top: GAF interaction with the *hsp70* promoter region leads to chromatin decondensation and a positioning of RNA polymerase II (RNAP II), which then remains associated with the 5' region of *hsp70* in a transcriptionally paused state (in complex with DSIF and NELF). At the bottom: As a result of the interaction with HSF, protein kinase P-TEFb binds to the transcription complex, the CTD of RNA polymerase II is phosphorylated, and NELF dissociates from the complex. Transcription consequently proceeds to elongation. The FACT and Spt6 complexes modify nucleosomes. Based on [30, 86, 87].

addition to the above factors, transcription of the *hsp70* genes involves the FACT (facilitates chromatin transcription) complex, which plays a role in nucleosome modification; topoisomerase I, which releases strain in the DNA double helix during transcription; and poly(ADP-ribose) polymerase (PARP), which is probably responsible for chromatin decondensation and nucleoplasm compartmentalization in the region of transcription [30–33]. It should be noted that the above mechanism activating transcription of the *hsp70* genes is not universal for all *Drosophila* HS genes. For instance, the promoters of *hsp68* and *hsp83* do not contain GAGA sites, nor do they interact with GAF, but the level of their transcription (especially that of *hsp68*) substantially increases in HS.

Studies with nonmodel organisms showed that, in contrast to HSF, GAF is not universally involved in *hsp70* transcription in all Diptera. The *hsp70* promoters of *Stratiomys singularior* (family Stratiomyidae) and *Ceratitis capitata* (Tephritidae) contain canonical HSE sequences, but do not have canonical GAGA sites, nor does the *S. singularior* promoter interact with GAF in vitro. Activity of the *S. singularior hsp70* promoter inserted in a reporter construct in *D. melanogaster* cells is lower than that of its *D. melanogaster* counterpart, the difference being tenfold in cultured Schneider 2 cells and 100-fold in vivo (in transformed embryos and transformed strains). The finding indicates that different mechanisms regulating transcription in stress formed upon divergence of large taxa (at the level of families) in Diptera. The promoters of *hsp83* in Diptera and *Hsp70* in mammals are far more conserved and universal than the *hsp70* promoters of Diptera [25, 26].

The constitutive HSE-binding factor (CHBF, or the Ku autoantigen) is constitutively bound to the promoter region of the *hsp70* genes at normal temperatures [89, 90]. The Ku autoantigen is a heterodimer and consists of 70- and 86-kDa subunits. The Ku autoantigen is known to act as a transcription factor that is involved in tRNA synthesis by RNA polymerase III and as an essential component of DNA repair and V(D)J recombination [91]. Yolk protein factor 1 (YPF1) is a *Drosophila* CHBE homolog [92]. CHBF (or YPF1 in *Drosophila*) is bound to the *hsp70* promoter at normal temperatures. The interaction weakens in HS, and CHBF is partly replaced by HSF1. An opposite process takes place during recovery after HS. When cells are

exposed to certain chemical agents, such as sodium arsenite and cadmium chloride, synthesis of the constitutive HSP Hsc70 increases, but Hsp70 induction is lacking, and CHBF remains associated with the HSE. Likewise, mild HS (41°C) causes HSF1 trimerization in mammalian cells, but does not lead to Hsp70 induction, and CHBF similarly remains associated with the *Hsp70* promoter. When HS is more severe (>42°C), *Hsp70* expression is triggered, and CHBF leaves the promoter and is replaced with HSF1. The CHBF-mediated regulation is specific to the *hsp70* promoter. CHBF was reported to directly interact with HSF1 to displace it from the transcription complex [89]. CHBF binds to DNA in a sequence-specific manner, as is evident from the fact that CHBF recognizes certain nucleotide sequences, such as the *hsp70* promoter.

Many other factors may be involved together with the above ones in activating transcription of the HS genes in various organisms. For instance, the molting hormone ecdysone induces low-molecular-weight HSPs in *Drosophila* [93, 94]. The promoters of the mammalian *Hsp70* genes bind with Sp1, which is absolutely essential for their basal transcription [28], and with the CCAAT-binding protein (CBP) and the CCAAT box transcription factor (CTF), which are also involved in basal synthesis of the Hsp70 mRNA [27]. The promoters of mammalian *Hsp70A1A*, *Hsp70A1B*, and *Hsp90* harbor recognition sites for NF-κB and STAT-3, which mediate the regulation of Hsp70 expression by cytokines, such as tumor necrosis factor α (TNFα) and interferon γ [29].

SYSTEM MAINTAINING PROTEIN HOMEOSTASIS IN THE ENDOPLASMIC RETICULUM AND MITOCHONDRIAL MATRIX

An increase in the cytosolic concentration of denatured proteins activates the system that involves HSF and additional factors and is responsible for transcription of the classical HS genes. While HS and other nonspecific exposures affect all systems of the cell, certain stress factors act selectively at the level of protein folding in the endoplasmic reticulum and mitochondria. The factors include glucose deficiency, N-glycosylation inhibitors, and calcium chelators. The cell consequently needs a system to monitor the protein status in the endoplasmic reticulum and mitochondria. The system is known as the unfolded protein response (UPR) system [95]. The system controls predominantly the genes for chaperone proteins that are transported after synthesis into the endoplasmic reticulum (ER chaperones) and mitochondria and are known as glucose-regulated proteins (GRPs) [6]. On the other hand, activation of the UPR system can induce apoptosis when stress is so intense that its consequences are impossible to compensate for via chaperone production.

The system that responds to protein denaturation in the endoplasmic reticulum (UPR^{ER}) is as conserved as the system that regulates transcription via HSFs. Its main element is inositol-requiring enzyme 1 (Ire1), which was comprehensively studied in yeasts, plants, and mammals and was found to act as a receptor that perceives stress signals at the endoplasmic reticulum level. Ire1 is a transmembrane protein and occurs in the membrane of the endoplasmic reticulum. Structurally, Ire1 includes three functional domains: a stress-sensitive intraluminal domain acts as a receptor, and two cytosolic domains are catalytic, possessing protein kinase and endoribonuclease activities, respectively. Two Ire1 isoforms, IRE1α and IRE1β, were identified in mammals. In the absence of stress, IRE1α activity is blocked by interactions of the intraluminal domain with HSPA5/BiP (binding immunoglobulin protein, which belongs to the Hsp70 family and is localized in the endoplasmic reticulum). In stress, IRE1α and BiP dissociate because unfolded proteins appear in the endoplasmic reticulum lumen and BiP more efficiently interacts with them. Thus, several features are common for the mammalian HSF and UPR systems as far as their activation is concerned. In either case, chaperones act as negative regulators and interact with the target factor (NSF or Ire1) to produce a stress-sensitive complex that responds to generation of denatured proteins. On the other hand, activation of yeast Ire1 and mammalian IRE1β is mostly due to direct interactions with unfolded proteins, rather than to dissociation from BiP [95]. Once activated, Ire1 undergoes oligomerization and autophosphorylation, which change the conformation and activate the endoribonuclease domain. The endoribonuclease then performs noncanonical splicing of the mRNA of a transcription factor that is responsible for transcription of the UPR target genes. The factor was described as Hac1 in yeasts, bZIP60 in plants, and Xbp-1 in *Drosophila* and mammals. The mRNAs of the factors occur in the cytoplasm in an inactive (untranslatable) form in normal conditions. When Ire1 is activated, its endoribonuclease excises the intron that contains a translational attenuator from the target mRNA [96, 97]. The Hac1/bZIP60/Xbp-1 mRNAs are translated after splicing, and their protein products are transported into the nucleus and bind to the regulatory regions of target genes to increase the intensity of their transcription. Yeast Hac1 recognizes a nucleotide sequence known as the unfolded protein response element (UPRE). The UPRE is an imperfect palindrome with a 1-bp spacer, CAGCGTC [97]. UPRE-like elements are involved in regulating seven yeast genes: *Kar2p*, *Lhs1*, and *Cer1p* of the *Hsp70* family; *Pdi1p; Eug1p;* and *Fkb2p*. The *Kar2p* promoter contains not only UPRE, but also HSE sequences and is activated by HSF in HS as well [98].

Apart from Xbp-1, other transcription factors—ATF4, ATF6, NF-Y, YY1, and YB-1—are involved in regulating the genes for endoplasmic chaperones in mammals [99]. To allow transcription in response to activation of the mammalian UPR^{ER} system, the promoter region of a target gene should contain two alternative sequences

known as the endoplasmic reticulum stress elements, ERSE and ERSE-II. The ERSE has a tripartite structure with the sequence 5'-CCAAT(N_9)CCACG-3', where N_9 is a 9-bp GC-rich region with the consensus CGGC-GGCGG [99–101]. The human *HspA5* promoter contains three ERSEs upstream of the TATA box at positions –126, –94, and –61 relative to the transcription initiation site. The *Grp94* promoter harbors two ERSEs in an inverted orientation at positions –195 and –137 of the transcription start, while a third ERSE is at position –72. ERSE-II, which has the sequence ATTGG(N)CCACG, was found in the promoters of *Herp* and several other genes [101].

In addition to splicing the Xbp-1 mRNA, the *Drosophila* and mammalian Ire1 proteins cleave several mRNAs by recognizing and hydrolyzing consensus sequences that code for an endoplasmic reticulum localization signal, thus preventing the synthesis of proteins transported into the endoplasmic reticulum. This mechanism blocks the protein transport into the endoplasmic reticulum until normal folding conditions are restored. When stress is too severe or persists for too long, Ire1 can induce apoptosis rather than an adaptive response by cleaving certain anti-apoptotic pre-miRNAs involved in suppressing the caspase-2 gene [95].

In mammals, the regulation of the UPR system involves not only IRE1, but also protein kinase R-like endoplasmic reticulum kinase (PERK) and ATF6. Like IRE1 PERK and ATF6 are transmembrane proteins and are activated in response to the stress factors that affect the functions of the endoplasmic reticulum. Protein kinase PERK attenuates translation by phosphorylating eIF2α. An overall decrease in translation, as well as Ire1-dependent cleavage of several mRNAs whose products are transported into the endoplasmic reticulum, decreases the total amount of proteins that are capable of denaturation and aggregation and thus reduce the stress load on the chaperone system. In addition, eIF2α phosphorylation selectively increases translation for ATF4, which activates transcription of UPR target genes [102]. Activated ATF6 is transported into the Golgi complex and undergoes specific proteolysis with a release of the cytosolic domain, which is transferred into the nucleus and binds to the regulatory regions of target genes to activate their transcription [95]. The regulation of the mammalian UPR is illustrated in Fig. 4.

Mitochondrial protein denaturation is caused by the same stress factors that damage proteins located in the endoplasmic reticulum. Several genes are activated in response, and their products are transferred into the mitochondrial matrix to facilitate restoration or degradation of denatured mitochondrial proteins. The stress response of this type is known as UPR^{mt} and is far less clearly understood than the regulation of synthesis of the classical HSPs and endoplasmic chaperones. It is known that protease ClpP is involved in degrading denatured mitochondrial proteins. The resulting peptides are transferred from the matrix into the cytosol. In *Caenorhabditis elegans*, the process depends on HAF1, which belongs to the ABC transporter family and is related to mammalian TAP1, which transports antigenic peptides into the endoplasmic reticulum. A vertebrate analog of HAF1 is unknown. In *C. elegans*, the release of peptides from mitochondria activates the ATFS-1 (activating transcription factor associated with stress 1) and DVE1 (defective proventriculus) transcription factors and increases transcription of the genes for ClpP, GroEL, and mitochondrial Hsp70. In mammals, the peptide transport from mitochondria into the cytosol activates PKR and JNK. The mechanism whereby mitochondrial peptides are recognized in the cytosol is unknown. Then JNK phosphorylates c-Jun, which is involved in inducing transcription of mitochondrial chaperone genes, while PKR phosphorylates eIF2α and thereby decreases the overall translation level [103].

CHANGES IN THE GENERAL MECHANISMS OF TRANSCRIPTION AND TRANSLATION IN STRESS

Activation of the HS genes is accompanied by repression of virtually all other genes in HS and exposure to certain other stress factors. The repression can be observed on salivary gland polytene chromosomes of *Drosophila* larvae and is seen as a regression of the puffs that were active at the given developmental stage prior to stress. Ribosomal and histone genes are exceptions, although splicing of their mRNAs is suppressed in HS. Nuclear tRNA and mitochondrial RNA genes are transcribed at the same intensity as before stress [104, 105]. Polysomes existing in the cytoplasm are decomposed soon (within 5–10 min) after an increase in temperature, and new polysomes form on HS mRNAs arriving from the nucleus. Disruption of the existing polysomes is not associated with competition for ribosomes between existing and newly synthesized mRNAs. This is evident from the fact that new mRNAs are not synthesized when α-amanitin or actinomycin D is used simultaneously with an increase in temperature to block protein synthesis, while old polysomes are still degraded. Some preexisting polysomes persist in the cytoplasm, but translation on them is blocked. Normal protein synthesis is resumed on these polysomes when the temperature decreases [106].

Thus, hyperthermia and other stress actors suppress transcription of the majority of genes that are active in normal conditions, inhibit translation of templates preexisting in the cytoplasm, and induce overexpression of a specific HS gene set and intense translation of their mRNAs while overall genome activity is repressed. The aim of these changes is to prevent the production of new proteins, which will be damaged in HS, until the cell is again in normal conditions and is status is normalized owing to Hsp accumulation.

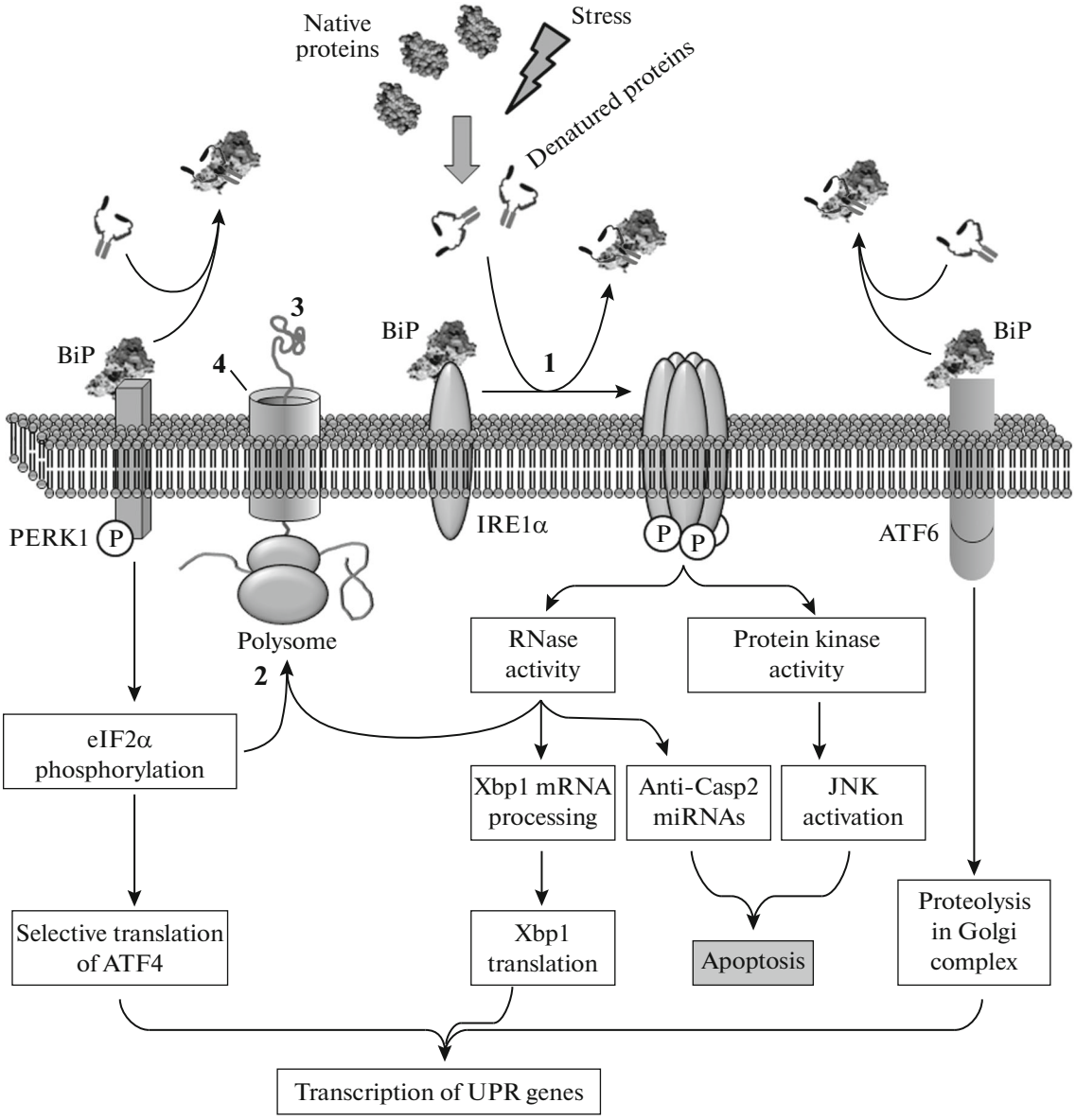

Fig. 4. Transcriptional regulation of the genes for glucose-regulated proteins, which are responsible for maintaining protein homeostasis in the endoplasmic reticulum (UPR^{ER}). (1) An increase in unfolded protein concentration in the endoplasmic reticulum lumen leads to dissociation of the Ire1α–BiP complex and oligomerization of Ire1α. Then Ire1α is autophosphorylated and triggers several signaling cascades, including a noncanonical splicing of the Xbp1 mRNA, cleavage of mRNAs for proteins targeting to the endoplasmic reticulum, and induction of apoptosis in severe stress via JNK phosphorylation and hydrolysis of antiapoptotic pre-miRNAs. (2) Translation is attenuated as a result of eIF2α phosphorylation and cleavage of the mRNAs specific to the system of protein translocation into the endoplasmic reticulum. (3) A protein being transferred into the endoplasmic reticulum during translation and (4) a translocon. Based on [95, 101].

Selective transcription of the HS genes in stress is ensured by changes in the dynamics of CTD phosphorylation of the RNA polymerase II large subunit in mammals. Dephosphorylated CTD is necessary for assembly of the initiation complex, while CTD phosphorylation is required for the transition to the elongation stage. CTD phosphorylation/dephosphorylation is performed by various protein kinases, including DNA-PK and MAPK, and CTD phosphatase FCP-1. Experiments with HeLa cells showed that HS leads to CTD hyperphosphorylation, which is driven by stress-induced CTD kinase and MAPK p42/p44. CTD kinases that are active in the cell in normal conditions are inactivated. FCT-1 is inactivated and denatured at the same time [107, 108]. It seems that RNA polymerase II with the hyperphosphorylated CTD loses its affinity for the promoters of "normal" genes and increases its efficiency in transcription from the HS gene promoters. There are data that HSF1 can act in HS not only as an activator, but also as a transcriptional

repressor of certain mammalian genes, for instance, by binding to the 5'-UTR of the *TNFα* gene [109].

Another hypothetical mechanism of transcriptional suppression in HS suggests that RNA polymerase II interacts with several noncoding RNAs whose synthesis is activated at higher temperatures. RNAs synthesized from Alu repeats, which are transcribed by RNA polymerase III, act as these RNAs in human cells. Alu RNAs occur in the nucleus at a relatively low level in normal conditions, and their concentration increases 40-fold in HS. According to a model advanced, Alu RNAs directly bind with RNA polymerase II in stress and alter its interaction with the promoters that do not have HSEs and do not bind with HSF1 [110].

Pre-mRNA splicing is suppressed in HS. Inducible HS genes lack introns in the majority of organisms, and splicing is therefore not required for their mRNAs. HSPs (Hsp104 together with Hsp70 and Hsp40) are necessary for the restoration of splicing machinery after HS in yeasts [111].

Translation of the majority of cell mRNAs is blocked in HS and virus infections as a result of changes in the translation initiation system. Generally similar mechanisms are responsible for overall translational suppression in virus infections and HS. Activation is observed for PKR in virus infections and HS, protein kinase HRI (heme-regulated inhibitor of translation) in hypoxia and HS, and PERK in dysfunction of the endoplasmic reticulum. The enzymes phosphorylate the α subunit of the eukaryotic translation initiation factor 2 (eIF2) at Ser51 [112]. Phosphorylated eIF2 loses the capability of exchanging GDP for GTP with the help of eIF2B, which forms a tight complex with eIF2. Translation initiation is thus blocked. The eIF2α phosphorylation level increases two- to threefold in HS [113–115].

Another mechanism of translational suppression in stress is based on dephosphorylation of the eIF4E-binding proteins (eIF4E-BP1, eIF4E-BP2, and eIF4E-BP3), which block the interaction of the cap-recognizing factor eIF4E with eIF4G, but not with the cap. Dephosphorylated eIF4E-BP has higher affinity for eIF4E, and their interaction decreases the translation intensity on the majority of polysomes active in normal conditions. Dephosphorylation of eIF4E also takes place in HS and virus infection to decrease its affinity for the cap [116].

The overall translation level in the cell decreases substantially (up to tenold) in virus infections and HS, and this decrease is accompanied by intense selective translation of virus RNAs or HS mRNAs. This selectivity is ensured by specific sequences contained in virus and HS templates. As is long known, HS mRNAs and the Hsp70 mRNA in particular differ in 5'-UTR structure from mRNAs synthesized at normal temperatures. The 5'-UTR of the Hsp70 mRNA is approximately 250 nt and consists almost exclusively of purines in the majority of organisms (e.g., in mammals and Diptera). According to the available data, translation of the human Hsp70 mRNA in stress resembles translation of picornavirus mRNAs or adenovirus late mRNAs. The Hsp70 mRNA is presumably translated in HS via a mechanism known as jumping, shunting, or hopping [117]. The gist is that the 40S ribosome subunit does not scan the total 5'-UTR during translation initiation, but skips a substantial region. The mechanism is determined by specifics of the 5'-UTR secondary structure. Usual translation with scanning the total 5'-UTR occurs in normal conditions, while the jumping mechanism is utilized in virus infection and HS for templates that contain a certain secondary structure in the 5'-UTR. The jumping mechanism does not require complete assembly of the complex of translation initiation factors, thus allowing the Hsp70 and adenovirus mRNAs to be translated selectively and at a high rate. The 5'-UTR of the adenovirus Ad5 mRNA contains three regions (C1–C3) that are complementary to two regions of the 3'-terminal hairpin of the 18S rRNA of the 40S ribosome subunit. A combined deletion of the C2 and C3 regions causes a 20-fold decrease in translation efficiency, directly implicating the regions in translation. The 5'-UTR of the Hsp70 mRNA was also found to harbor a sequence complementary to the 3'-terminal hairpin of the 18S rRNA, and its deletion decreases the translation efficiency in HS more than threefold. The molecular mechanism that sustains translation initiation on adenovirus and Hsp70 mRNA is not completely understood, but is possibly similar to the prokaryotic initiation utilizing the Shine–Dalgarno sequence [117]. Experiments have not yet been carried out to directly detect the interaction of the 5'-UTRs of the Hsp70 and adenovirus late mRNAs with rRNA.

It was shown that both *Drosophila* and human Hsp70 mRNAs are translated via an internal initiation mechanism, which is well known for various virus RNAs and is mediated by the so-called internal ribosome entry site (IRES) present in their 5'-UTRs [118, 119]. The IRES binds with the preinitiation complex in the 40S ribosome subunit associated with eIF2 and eIF3 and positins the mRNA start codon via a cap-independent mechanism without a preliminary 5'-UTR scanning, which requires eIF4G and eIF4A. The Hsp70 5'-UTR added to a reporter construct increased its translation in HS by a factor of 100, the translation efficiency being similar to that observed with the classical picornavirus IRES [118].

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