Regulation of the heat shock response by heat shock transcription factors

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Abstract

The heat shock response is characterized by a rapid and robust increase in heat shock proteins upon exposure to protein-damaging stresses. This evolutionarily conserved cellular protection mechanism is primarily regulated at the level of transcription. In bacteria, heat shock-induced transcription is regulated by the activation of σ^{32} factor, whereas eukaryotes utilize heat shock transcription factors (HSFs) that bind to specific heat shock elements (HSEs) within the promoters of their target genes. Unlike yeasts, nematodes, and fruit flies, which have a single HSF, vertebrates and plants have an entire HSF family. In addition to stress-induced activation, some members of the HSF family are also activated under non-stressful conditions, including development and differentiation. The activity of HSFs is under post-translational control, requiring trimerization, DNA binding, and hyperphosphorylation. The interplay between different family members and other interacting proteins adds further complexity to HSF-mediated transcription. Here, we summarize the current knowledge of the transcriptional regulation of the heat shock response, highlighting recent advances in exploring the multi-faceted nature of heat shock transcription.

1 Preface

Maintenance of cellular protein homeostasis requires the proper function of molecular chaperones. Molecular chaperones comprise a great variety of proteins that are essential for protein folding and translocation across cellular compartments, assembling multi-protein complexes, preventing aggregation, and directing misfolded and short-lived proteins to degradation by the proteasome. Protein-damaging stresses, including heat shock, induce the expression of a subgroup of molecular chaperones, called heat shock proteins (Hsps), which consist of several protein families designated by their molecular weight, such as the Hsp90, Hsp70, Hsp60, and the small Hsp (sHsp) families. Upon stress, the Hsps prevent protein unfolding and aggregation, thereby maintaining the critical cellular structures and functions and protecting against apoptotic or necrotic cell death. The expression of Hsps is regulated by multiple mechanisms, among which the transcriptional regulation is most prominent. The transcriptional regulation of the heat shock response

is conserved throughout the eukaryotes, where the different members of the HSF family share homologous functional domains and act mainly as transcriptional activators. In addition to stress stimuli, some HSFs respond to other signals and have distinct targets from the classical heat shock genes. Consequently, HSFs are involved in a number of vital physiological processes, such as development, differentiation, and regulation of longevity. The central role of HSF-mediated transcription in several cellular processes underlines the importance of understanding the functions of HSFs in normal physiology and disease conditions.

2 Transcriptional regulation of the heat shock response in bacteria

The function of Hsps and the presence of heat-shock inducible transcription are strikingly well conserved throughout evolution. Despite certain analogies, however, the transcriptional regulatory mechanisms are distinct in prokaryotes and eukaryotes. The regulation of the bacterial heat shock response is best characterized in *Escherichia coli* where the expression of Hsps, including DnaK (prokaryotic Hsp70), DnaJ (Hsp40), GrpE, GroEL (Hsp60), and GroES (Hsp10), is regulated by the product of the *rpoH* gene, i.e. the stress-inducible σ^{32} subunit of RNA polymerase (Fig. 1A; Grossman et al. 1984; for review see Arsène et al. 2000). Under non-stressful conditions, σ^{32} is maintained at a low level due to its rapid turnover, but upon exposure to heat shock, the concentration of σ^{32} is greatly increased through enhanced synthesis and increased stability, resulting in preferred transcription of σ^{32}-dependent heat shock genes (Straus et al. 1987; for review see Yura and Nakahigashi 1999; Arsène et al. 2000).

The σ^{32}-mediated transcription is controlled by a negative feedback system. The accumulating DnaK-DnaJ-GrpE chaperone machinery binds to σ^{32} and inhibits its activity (Tilly et al. 1983; Straus et al. 1990; Tomoyasu et al. 1998). Moreover, binding of DnaK-DnaJ to the σ^{32} promotes its degradation by the ATP-dependent metalloprotease FtsH (Tatsuta et al. 1998). Therefore, availability of DnaK/DnaJ is a direct sensor of cellular stress and a regulator of heat shock transcription (Tomoyasu et al. 1998). In addition to the central role of σ^{32}-mediated regulation of the heat shock response, which is well conserved in Gram-negative bacteria (for review see Yura and Nagahigashi 1999), distinct regulatory mechanisms have been characterized in other bacteria. For example, in the Gram-positive bacteria *Bacillus subtilis*, the HrcA repressor regulates major Hsps by binding to negatively acting CIRCE elements (Fig. 1B; for review see Yura and Nagahigashi 1999). Interestingly, the folding of HrcA is facilitated by the GroE chaperonin system. In response to increased protein damage, the GroE folding machinery is occupied and folding of the HrcA repressor is stalled (Mogk et al. 1997). Thereby this regulatory system also relies on the availability of Hsps, providing a direct sensing mechanism for protein misfolding.

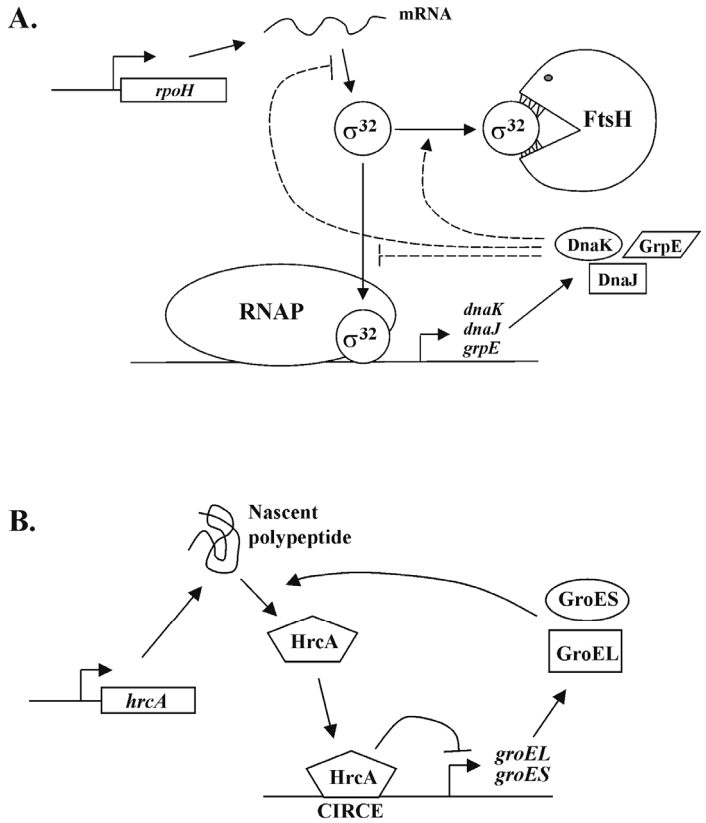

Fig. 1. Regulatory mechanisms of the prokaryotic heat shock response. A. In Gram-negative bacteria, the heat shock response is regulated by the σ^{32} factor. Accumulation of the heat shock proteins DnaK, DnaJ, and GrpE provides a negative feedback mechanism by inhibiting σ^{32} translation and association with the core RNA polymerase and by promoting σ^{32} degradation by FtsH. B. Regulation of the heat shock response by CIRCE elements and the HrcA repressor. Negative feedback regulation is provided by GroE chaperonins, which facilitate the folding of HrcA.

3 Regulation of the eukaryotic heat shock response via heat shock elements

Protein-damaging conditions have profound effects on eukaryotic transcription. While transcription is generally inhibited, the transcription of genes encoding Hsps can be increased more than 100-fold upon heat shock (Gilmour and Lis 1985). This robust activation of gene expression was initially reported by Ferruccio Ritossa (1962), who observed induction of specific chromosome puffs in the polytene chromosomes of *Drosophila buschii* larval salivary glands accidentally

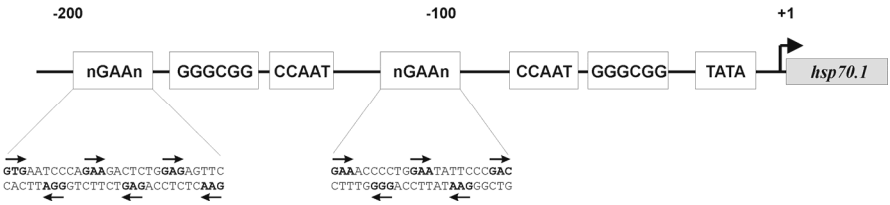

Fig. 2. The human *hsp70.1* promoter contains two stress-responsive heat shock elements. The human *hsp70.1* promoter contains a proximal and distal HSE with five and six inverted nGAAn repeats, respectively. The GC-, CCAAT-, and TATA-boxes are involved in constitutive *hsp70.1* expression and maintenance of chromatin accessibility.

exposed to elevated temperatures. Ten years later, Tissieres and coworkers discovered the stress-induced synthesis of Hsps (Tissieres et al. 1974), and after another decade, the heat shock element (HSE), a specific DNA sequence responsible for the transcriptional activation of heat shock genes, was identified (Pelham 1982). The consensus HSE consists of contiguous inverted pentameric repeats of the sequence nGAAn (Amin et al. 1988; Xiao and Lis 1988; Kroeger and Morimoto 1994). For example, the human *hsp70.1* promoter contains two HSEs, of which the proximal element contains five and the distal element six pentameric units (Fig. 2; Greene et al. 1987; Wu et al. 1987; Abravaya et al. 1991a, 1991b). In addition to the HSEs, the *hsp70.1* promoter contains binding sites for other transcriptional regulators, such as the GC and CCAAT boxes, which are involved in the basal expression of *hsp70.1* and in the maintenance of accessible chromatin architecture of the promoter (Williams et al. 1989; Williams and Morimoto 1990; Landsberger and Wolffe 1995; Bevilacqua et al. 1997; Imbriano et al. 2001).

4 Heat shock factors constitute a conserved family of transcriptional regulators

The eukaryotic transcription factors that bind to HSEs and activate transcription are called heat shock transcription factors or HSFs (for review see Wu 1995; Pirkkala et al. 2001). In yeast and fruit fly, a single HSF is responsible for HSE-mediated transcription (Sorger and Pelham 1988; Wiederrecht et al. 1988; Clos et al. 1990), but in vertebrates, the HSF family consists of several members sharing functional and structural properties, such as domains involved in DNA binding and oligomerization (Fig. 3). In mammals, three HSF family members, HSF1, HSF2, and HSF4, have been found, whereas avian species have an additional heat shock factor, HSF3 (Rabindran et al. 1991; Sarge et al. 1991; Schuetz et al. 1991; Nakai and Morimoto 1993; Nakai et al. 1997). The diversity of mammalian HSFs is increased by alternative splicing of HSF1, HSF2, and HSF4 (for review see Pirkkala et al. 2001). Despite the family name, not all heat shock factors are stress responsive. Among the vertebrate HSFs, only HSF1 and the avian-specific HSF3 have conclusively been shown to be activated by stress and to be essential for the

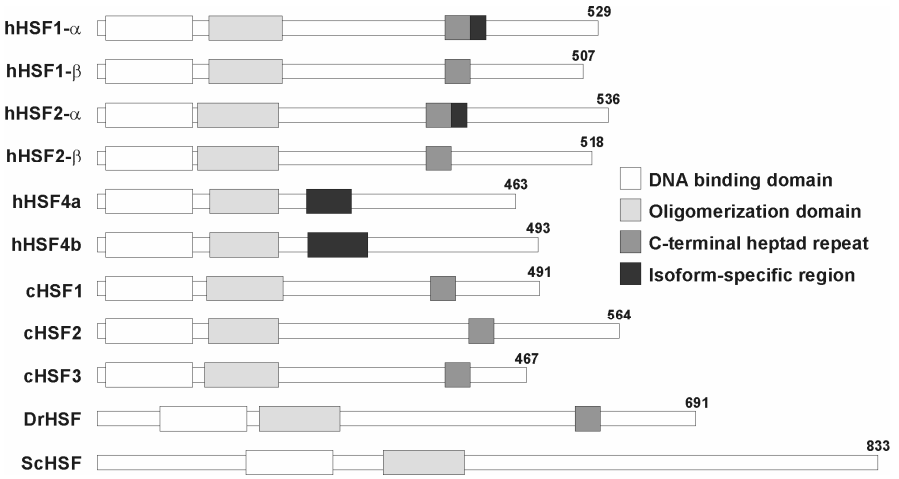

Fig. 3. Common structural features of heat shock transcription factors. A schematic presentation of conserved domains of HSFs (h, human; c, chicken; Dr, fruit fly; Sc, budding yeast). All HSFs have a DNA-binding domain and N-terminal heptad repeat involved in trimerization. HSF4 and ScHSF lack the C-terminal heptad repeat, which negatively regulates trimerization. Mammalian HSF1, HSF2, and HSF4 are expressed as two alternatively spliced isoforms. The number of amino acids in each HSF molecule is indicated.

transcriptional regulation of the heat shock response (Nakai et al. 1995; McMillan et al. 1998; Tanabe et al. 1998; Xiao et al. 1999). It has been postulated that the HSFs that are refractory to stress stimuli would regulate transcription under other circumstances, such as during development and differentiation, and possibly have target genes distinct from the classical heat shock genes (for review see Pirkkala et al. 2001). Recent studies, however, indicate that there is more interplay between the classical and non-stress-responsive HSFs than originally anticipated (Alastalo et al. 2003; He et al. 2003). In addition to the transcriptional activators of the HSF family, the HSF4a isoform lacks transcriptional activity and has been suggested to function as a repressor of transcription (Nakai et al. 1997; Tanabe et al. 1999).

Although heat shock transcription in plants is under the control of HSFs, the regulatory mechanisms differ substantially from those in animal species. A striking property of plant HSFs (Hsfs) is their great number and complexity. For example, in *Arabidopsis thaliana*, 21 genes coding for Hsfs have been found (for review see Nover et al. 2001). Based on structural features, these Hsfs can be assigned to three classes (A, B, and C) and 14 groups. Although the functional significance of the multitude of plant Hsfs is not fully understood, studies on tomato, which to date provides the best characterized Hsf system, have revealed novel regulatory principles that diverge between plant and animal HSFs. Unlike the stress-responsive HSFs in animals, which are mainly regulated post-translationally, certain tomato Hsfs display stress-inducible expression (for review see Nover et al. 2001). In addition, Hsfs are regulated hierarchically, as tomato