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How the Cell Copes with Stress and the Function of Heat Shock Proteins

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ABSTRACT

Virtually all cells, including the prokaryotic microorganisms and the highly differentiated eukaryotic cells in human tissues, contain a small set of normally silent genes that are rapidly activated by a heat shock that raises the temperature only 5 to 10% above that of the normal physiologic range for that organism. Concomitantly, many active genes are turned off. Other kinds of stress, such as exposure to alcohol or other organic agents, heavy metals, oxidants, and agents capable of perturbing protein structure, produce a similar response, and many of these activate the same set of genes. The proteins encoded by these stress-activated genes are called heat shock proteins (hsp). They are strongly conserved in structure among widely divergent biologic species, and many function as "molecular chaperones" by forming transient complexes with partially folded or misfolded polypeptides so as to prevent their irreversible denaturation. Most hsp are members of gene/protein families, and isoforms are frequently found under normal physiologic conditions in many compartments of the cell where they act also as chaperones, binding to a variety of polypeptides to facilitate folding, oligomerization, transport, metabolic activity, and degradation. Few of the polypeptide "targets" that complex with stress-induced forms of hsp have been identified, but a number of cellular components have been shown to be particularly stress sensitive. They include macromolecular complexes involved in the maintenance of chromosome replication and transcription, mRNA splicing, and ribosome assembly. Mitochondria and the intermediate filament network are also highly sensitive, whereas the protein synthetic machinery and vesicles of the secretory pathway are relative stable to physiologic stress. The factors regulating heat shock genes in the eukaryote are highly conserved among widely divergent species and include promoters consisting of arrays of short, inverted sequences in the DNA, called the heat shock element, and heat shock factors, which are large polypeptides that occupy these promoters soon after the cell senses the temperature shift. The sensor(s) that signal the cell to initiate binding of heat shock factors to the heat shock element, thereby activating gene transcription, have not been identified, but misfolded proteins are postulated to play a key role in this event. The response is also transient, and other factors down-regulate the system. Cells that have been mildly prestressed so that they contain significant levels of the hsp become tolerant to stress conditions that would normally kill the cell. In this way, organisms survive environmental conditions that might otherwise prove fatal. (***Pediatr Res* 36: 1–6, 1994**)

Abbreviations
hsp, heat shock protein
hsf, heat shock factor

Probably the most frequent calls received by a pediatrician concern a child with high fever, a condition generally symptomatic of illness. This febrile response is one of the early defenses against most infections, and yet these elevated temperatures are sufficient to cause irreparable harm to many cells in our body. Most cells escape heat-shock damage and death because they can sense the rise in temperature and respond in a way that protects them from irreversible heat-induced injury. Many of the biochemical mechanisms that lead to this protection were discovered only within the past 15 y, and this review summarizes those that occur primarily in the eukaryotic cell. A heat shock response is found also in the prokaryotes (1) but differs in some details from those of the nucleated cell and will not be described. The primary literature on heat shock is substantial, much of it appearing since 1985; however, comprehensive volumes and recent reviews are recommended to those desiring more specific citations (2–8).

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THE HEAT SHOCK PROTEINS

A comparison between normal and heat-shocked cells reveals a number of changes in their pattern of protein synthesis, which will vary somewhat depending on the

particular cell and the severity of the stress. Several of these alterations represent actual increases in levels of gene transcription and subsequent mRNA translation, whereas others result from posttranscriptional and post-translational modifications to mRNA and proteins present under normal physiologic conditions in the cell. Those proteins whose changes reflect newly activated genes are called hsp, and they probably number less than 50. The major ones have subunit molecular masses of 20–30 kD, 60–70 kD, and 80–100 kD and are now known to function as molecular chaperones (see below). Other hsp are enzymes that participate in metabolic pathways affected by the heat shock. For example, some glycolytic enzymes are induced, probably reflecting a shift from aerobic to anaerobic ATP generation, and several of the enzymes involved in the ATP-dependent ubiquitin pathway of protein degradation increase, reflecting the accumulation of denatured proteins in the heat-shocked cell. Enzymes involved in protein folding, *i.e.* the peptidyl proline isomerase (9), and in tyrosine phosphorylation (10, 11) are regulated by stress. A table in Nover's comprehensive review (2) lists about 30 stress-induced enzymes.

The function of the five or six major hsp was identified initially through the study of proteins (isoforms) closely related in structure to the hsp but synthesized constitutively under normal growth conditions. One of the first indications of an activity for an hsp in eukaryotic cells came from a serendipitous observation that a widely distributed ATP-dependent enzyme that uncoated clathrin baskets was immunologically related to hsp70 (12). Hsp70 was found to contain an ATP-binding site (13), and an interaction between ATP and hsp70 released the latter from an insoluble complex that forms in the nucleolus of a heat-shocked cell (14). The structure of hsp70's ATP-binding site, which occupies one domain of the protein, has recently been solved by x-ray diffraction analysis (15). Another clue to hsp70's function emerged from the observation that a previously studied protein, immunoglobulin heavy-chain binding protein (BiP), was an isoform of hsp70 (16). BiP had been shown to transiently bind the heavy chain of immunoglobulins in the endoplasmic reticulum of early B lymphocytes. Based on these observations, Pelham (17) suggested that hsp70 binds to unfolded proteins accumulating in a stressed cell and promotes their refolding and assembly.

The possibility that hsp could affect another polypeptide's conformation led investigators to examine hsp70 isoforms in yeast mutants defective in the transport of proteins into various cellular organelles (18). It turned out that some of these mutants were altered in genes belonging to a large family of yeast hsp70-like proteins. This yeast family is now known to consist of nine polypeptides closely related in structure but differing with regard to their cellular location, *i.e.* cytoplasm, nucleus, mitochondria, and secretory vesicles, and regulation, *i.e.* many are formed constitutively, others during a heat shock, and one during a cold shock (19). Constitutively synthesized forms of hsp70, called cognates or hsc70, were identified in many other kinds of cells and often were detected in complexes with a variety of other cellular proteins, including those involved in mitochondrial, nuclear, and lysosomal import. Similar kinds of protein complexes were found for hsp90 and included tubulin, vimentin, actin, calmodulin, and several protein kinases. An interaction of hsp90 with the intracellular hormone receptors has been studied in considerable detail, and models are proposed in which hsp90, hsp70, and an hsp56 form a "transportosome," which shuttles the receptor between the cell nucleus and cytoplasm (20). More recent data have shown that the low-molecular-weight hsp from chicken fibroblasts also forms complexes, in this case with actin (21).

All of these data ultimately led to the present concept that the major hsp function as molecular chaperones (22). In this role, they form transient complexes with a wide variety of cell polypeptides—many occurring normally during cell growth and division and others forming as a result of the heat shock. In the complex, the polypeptide is protected from misfolding and irreversible denaturation. Dissociation of the complex by ATP or another protein releases the polypeptide in a conformation that leads to a properly folded, biologically active protein. *In vitro* experiments with model proteins that have been denatured and renatured in the presence of purified chaperones have strongly supported the molecular chaperone model (23, 24). In addition, numerous genetic experiments, mostly in yeast and bacteria, provided data supporting this role for hsp (5). Complexes composed of these hsp chaperones could function also as "foldosomes."

Although many normal cellular polypeptides are known to be chaperoned by members of the hsp families, we still do not know the specific polypeptides in a stressed cell that are targets for hsp. Immunofluorescence studies have shown that hsp70 rapidly moves into the nucleus and the nucleolus of a stressed cell; thus, proteins functioning in these organelles are predicted to be targets. In addition, nascent polypeptides synthesized during a stress are found to be strongly associated with hsp (25).

THE HEAT SHOCK GENES

The report of new "puffs" in the polytene chromosomes of heat-shocked *Drosophila* larvae (26) was the first indication that this kind of stress could dramatically affect a cell's genome. The dominant puffs occurred at only a few places in the fly's chromosome and were shown to involve new RNA transcription. Gene activation by temperature change offered a facile system for investigating eukaryotic gene regulation, and much of the research in the heat shock field focused in this area. These studies led to the identification of the heat shock element, which consists of inverted repeats of the DNA sequence -nGAAn- (27) arrayed in sets of trimers located

at sites in the genome about 100–200 bp before the site for initiation of transcription of a heat shock gene, and the heat shock factor (hsf), which is a protein that binds specifically to this element (4). In most eukaryotic cells (the yeast *Saccharomyces cerevisiae* is the exception thus far) growing at normal temperatures, the heat shock promoters are unoccupied and hsf is present but cryptic. Very soon after a heat shock, however, hsf is activated and binds to the DNA, thereby stimulating transcription of the heat shock gene (Fig. 1). Eukaryotic hsf have been isolated, cloned, and sequenced from humans, mice, insects, plants, and yeast. Many of these contain more than a single gene encoding an hsf and, in the case of the mouse, there are two hsf that respond quite differently to temperature, *i.e.* one is inactive at normal temperatures but active at high temperatures, whereas the other has precisely the opposite properties (28). A similar situation exists in the chicken, where there is a third hsf expressed during development (29). Eukaryotic hsf have about the same subunit size, ranging from 50 to 70 kD, but are highly divergent in sequence except for a DNA-binding domain near the amino terminus and several repeats of hydrophobic amino acids containing a coiled-coil motif that functions as a trimerization domain (30). It is the hsf trimer that binds stably to the heat shock element of the DNA. The structure of the DNA-binding domain in the *Kluyveromyces lactis* hsf has been determined to 1.8 Å (31). A variation of a helix-turn-helix motif is postulated to account for protein-DNA contacts in this protein. A hydrophobic domain that is found in the C-terminal region of those hsf that are heat-shock inducible is postulated to act as a "suppressor" motif (Fig. 1), because hsf that lack this sequence form trimers in the absence of stress and activate the gene.

The cryptic hsf can be converted to active trimers by strong protein denaturants, but in the cell's cytoplasm they are stable, *i.e.* after repeated treatment of cells with cycloheximide to block protein synthesis, it is still possible to activate heat shock genes in these cells (32). More labile forms of hsf exist, however, because if cells are given cycloheximide and then a "mild" or intermediate heat shock, heat shock gene activation does not occur (33). When cDNA for either human or *Drosophila* hsf are inserted into appropriate expression vectors and transfected into cells, they are expressed in one of two forms. At normal temperatures, a monomeric form that does not bind to a DNA heat shock element can be isolated, but after heat shock, a DNA-binding protein with a larger molecular weight equivalent to an hsf trimer is found (34).

Cells in culture provide useful experimental systems to study heat shock gene regulation because temperatures can be readily shifted and controlled. In these cells, the application of a heat shock leads to an immediate induction of hsp. Heat shock gene transcription, however, is also negatively regulated, and in systems where expression of the hsp70 can be artificially adjusted, there is a strong correlation between levels of hsp70 and the extent of hsp gene activity (35, 36). Not all of the major hsp in eukaryotic cells are activated by the same stress, and, unlike the regulon system in *Escherichia coli* (5), they do not appear simultaneously. The pattern of hsp as well as the extent and duration of their synthesis varies greatly depending on the severity and length of treatment.

OTHER CHANGES IN THE STRESSED CELL

The rapid changes in gene activity after heat shock, although quite dramatic, actually represent only one of a number of very profound alterations made in a cell undergoing a heat shock response. Again, the extent of these modifications is closely correlated with the severity of the stress, but like the induction of new protein synthesis, most of these other events are transient and readily reversible as soon as the stress condition is removed. Many of the changes have been visualized by using the technique of immunofluorescence in which cells are fixed in a manner similar to that used for histologic staining. An antibody specific to a particular protein is

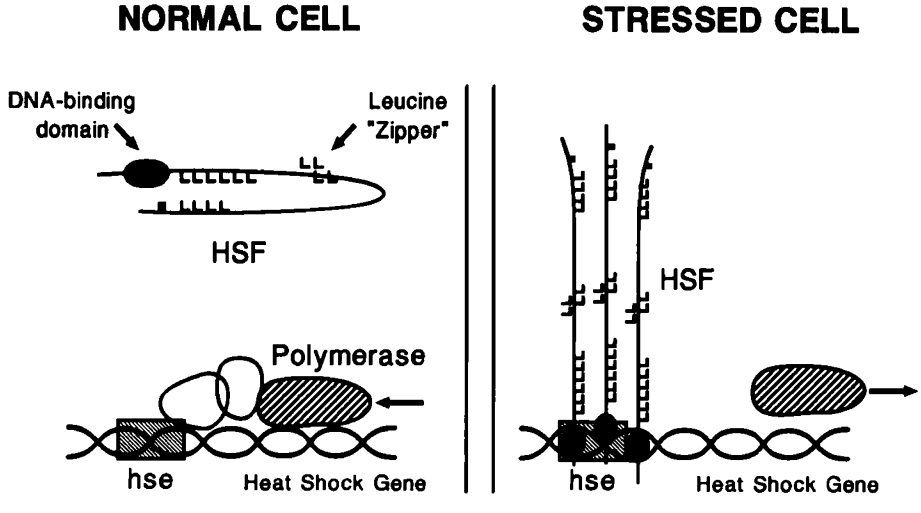

Figure 1. Different conformations of the heat shock factor and its interaction with the heat shock promoter (*hse*, heat shock element). Based on studies by Rabindran *et al.* (34).

applied, followed by an antiantibody carrying a fluorescent reagent. A more recent technique, which uses a video-enhanced optical system using differential contrast imaging, allows for real-time pictures of changes in live cells experiencing a heat shock (Fig. 2). All of these methods have been supplemented with biochemical measurements designed to follow macromolecular synthesis and degradation, alterations in energy metabolism, and variations in enzymatic activities.

The cell components that seem to be the most sensitive reside in the nucleus, where the chromatin and "factories" involved in mRNA maturation are localized. DNA structure and replication are very sensitive to stress; the latter stops abruptly, and the chromatin condenses as the histones are chemically altered, *i.e.* deubiquitinated and phosphorylated, in a manner similar to that noted in the early stages of mitosis. RNA synthesis continues but ribosomal RNA processing slows significantly, an indication that ribosomal assembly stops, and mRNA fail to be completely spliced. The nuclear membrane becomes more refractile, but it is unclear what this means biochemically, because the lamin network is not detectably modified. At more intense levels of stress, actin fibers are detected in the nucleus. In the cell cytoplasm, the intermediate filament network is highly stress sensitive and collapses to an unorganized array surrounding the nuclear membrane (37, 38). Concomitant with this disorganization is a fragmentation of the elongated, tubular mitochondria and their clustering around the cell's nucleus, where they appear as amorphous and structureless membrane "blobs." The microfilament network is altered, and bundles of actin fibers form with increasing amounts of stress (21). Microtubules seem to remain intact until stronger stress is experienced. The cellular secretory apparatus, *i.e.* the endoplasmic reticulum, Golgi stacks, and trans-Golgi vesicles, are unperturbed both structurally and functionally during mild, physiologic stress conditions. Similarly, the components of the signal transduction pathways are not highly sensitive to stress, as evidenced by little change in levels of inositol-Tris phosphate, calcium, pH, or ATP. Many of these components do respond and change after strong stress conditions have been applied to the test cell system.

From this abbreviated listing, one concludes that a stressed cell responds to the potential injury by two general mechanisms. One of these is the formation of a set of proteins that function as chaperones to prevent irreversible protein denaturation. The second is the shutting down of biosynthetic pathways containing components critical to the cell's ability to recover and return to normal metabolic state. Among these components are the DNA, ribosome, and spliceosome assembly systems and the mitochondria.

STRESS TOLERANCE

An important feature of the heat shock response is the protection from additional stronger stress conditions by a

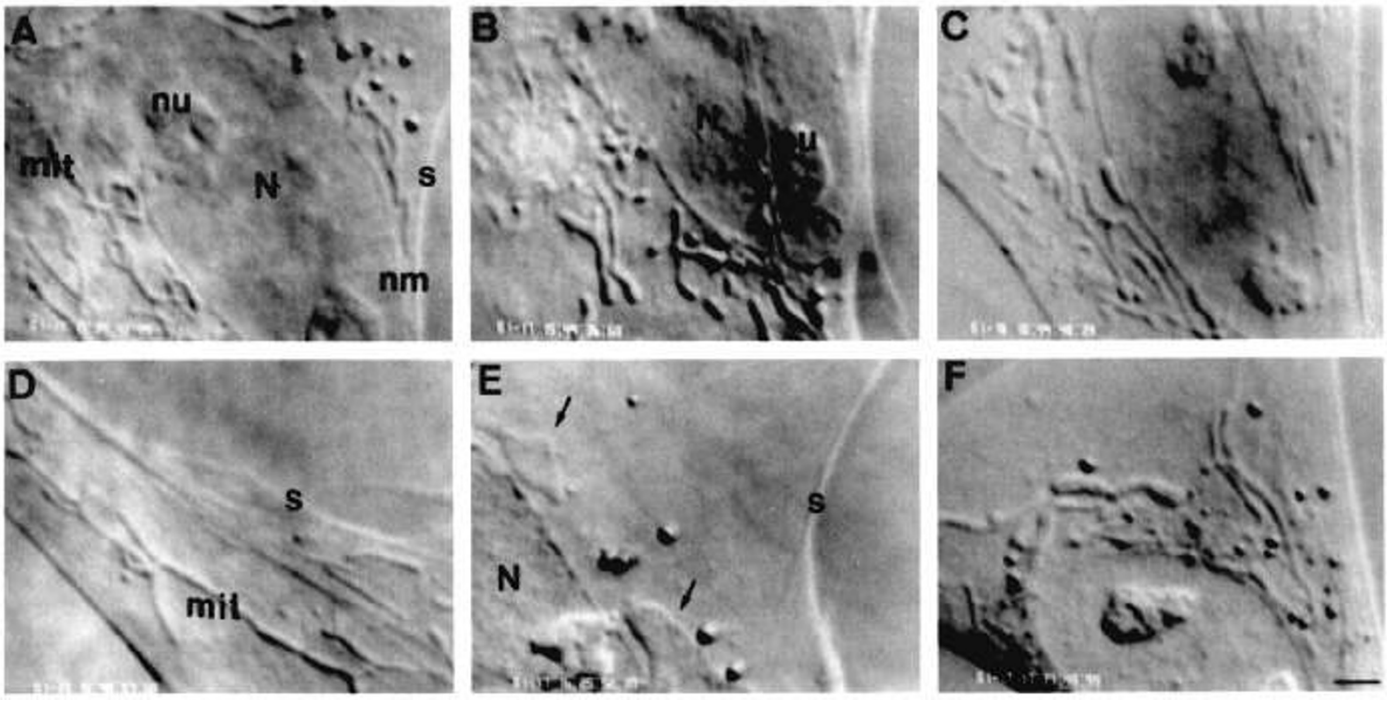

Figure 2. Video-enhanced differential interference contrast microscopy of normal, heat-shocked, and recovering chicken embryo fibroblast. Panels *A*, *B*, *D*, and *E* are of the same cell. Panels *A* (nuclear region) and *D* (cytoplasmic periphery) were taken at 37°C before heat shock. Panels *B* (nuclear region) and *E* (cytoplasmic periphery) were taken about 5 min and 40 min after increasing the temperature to 45°C, respectively. Panel *F* is a cell heat shocked for about 40 min and incubated 1 h at 37°C. Panel *C* is a cell from a separate coverslip that was heat shocked at 45°C for 1 h and allowed to recover overnight at 37°C before observation. *mit*, Mitochondria; *N*, nucleus; *nm*, nuclear membrane; *nu*, nucleolus; *s*, cell surface membrane. *Arrows* in panel *E* show fused mitochondria. Bar = 1 μm. From Collier *et al.* (37).

cell that has already experienced a stress (7). This phenomenon is referred to as stress-induced tolerance and is critical for those biologic systems where the level of stress gradually builds to extreme conditions, *i.e.* a plant exposed to increasing amounts of heat during the day. It is likely that the human febrile response also meets this criterion, inasmuch as a fever increases over a few days. In the absence of this "stress conditioning," the stronger stress would seriously injure the cell and lead to lethality.

Most experiments show a strong positive correlation between the extent of tolerance and the level of hsp; however, some data report that no tolerance occurs despite the presence of hsp and others indicate the existence of tolerance in the absence of hsp. An important feature of stress tolerance is the ability to induce tolerance by a stress condition different from that initially imposed on the cell. For example, a cell can acquire tolerance to a heat shock even if its initial exposure was to stress by a chemical such as arsenite or ethanol. The ability to establish a state of tolerance has recently been exploited as a potential mechanism to retain increased viability for cells destined to be used in organ transplants (39).

IMMUNOLOGIC EVENTS AND THE STRESS RESPONSE

The prevalence of increased levels of some hsp in stressed cells apparently leads to their recognition as foreign antigens by the immune surveillance system (40). One of the early indications of this phenomenon was the identification, in patients infected with the mycobacteria causing tuberculosis or leprosy, of a substantial fraction of cytotoxic T cells that recognized peptides with sequences found in the major prokaryotic cell protein, GroEL, an hsp (41). Subsequently, it was found that patients infected with the malarial parasite and with the fungal agent causing histoplasmosis had T cells recognizing peptides of the hsp70, and many of these were of the $\gamma\delta$ type (42). It is also intriguing that several human hsp70 genes are linked to the MHC locus (43), and one hsp70 family member is believed to be involved in antigen processing (44). In addition, there are several reported animal models in which injection of hsp leads to autoimmune symptoms. These results are surprising because the strong evolutionary conservation of these proteins would lead one to expect that an individual would normally have acquired tolerance to his or her own hsp. However, there is clearly the possibility that the isoform induced by stress would be sufficiently different antigenically to become a potential antigen in the mature individual.

SUMMARY

The strong evolutionary conservation of activities expressed in cells of widely different organisms during a heat shock and related kinds of stress indicates that this response is essential for survival of the organism. Within a relatively short time, scientists from many different disciplines discovered the essential biochemical mechanisms underlying the genetic changes first reported 30 y ago. The molecular components that regulate expression of heat shock genes are defined, and the general properties and the primary function of many hsp are known. A general view of hsp and their involvement in the cell is shown in Figure 3.

To be sure, much more information will be forthcoming and should further define the specific heat shock–sensitive targets protected by molecular chaperones. Perhaps most intriguing will be the identification of the sensor or sensors that tell the cell that it is experiencing

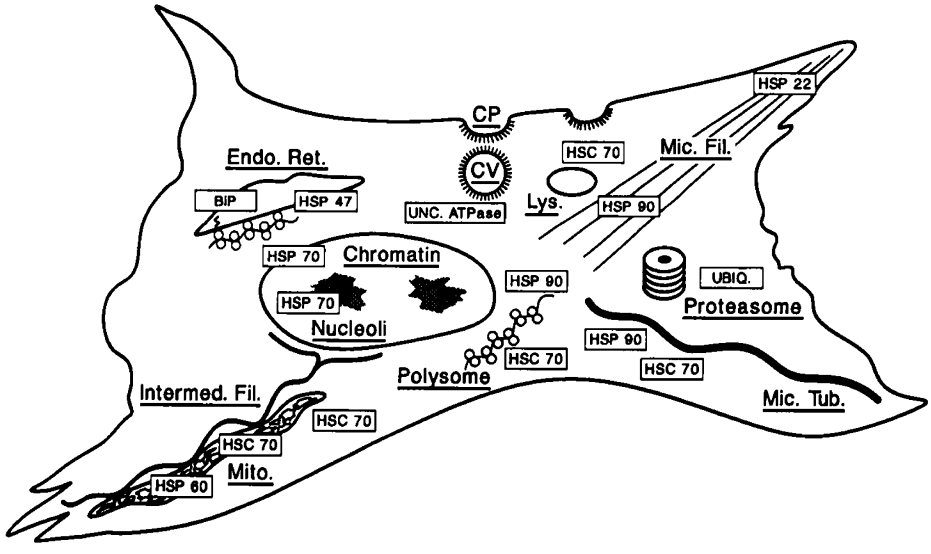

Figure 3. A heat-shock protein view of the cell. The major hsp are noted in boxes associated with various cellular organelles (nucleus, cytoplasm, mitochondria, endoplasmic reticulum, lysosome) and cytoskeletal elements (microfilaments, microtubules, and intermediate filaments). *CP*, coated pits; *CV*, coated vesicles; *UNC.*, uncoating; *UBIQ.*, ubiquitin; *Lys*, lysosome; *Mic. Fil.*, microfilaments; *Mic. Tub.*, microtubules; *Intermed. Fil.*, intermediate filaments; *Mito.*, mitochondria; *Endo. Ret.*, endoplasmic reticulum.

a stress and must respond. Currently, the components considered to be central to the sensing system are the hsf, hsp, and unfolded polypeptides generated by the stress (25, 30, 45–47). Protein complexes made up of these elements act to autoregulate heat shock genes, which contain specific promoters in their DNA sequences. Coupled with other events that shut down various metabolic activities, the cell is able to cope with the stress and establish homeostasis.

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