Cells in Stress: Transcriptional Activation of Heat Shock Genes

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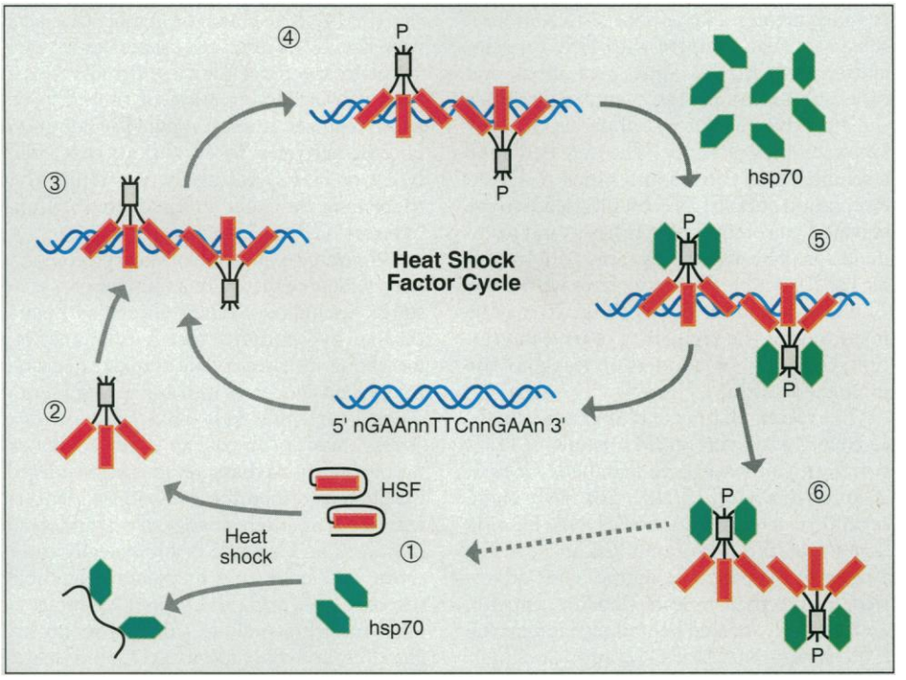
A major question in biology is how cells cope with rapid changes in their environment, such as exposure to elevated temperatures, heavy metals, toxins, oxidants, and bacterial and viral infections. It has become clear that all organisms share a common molecular response that includes a dramatic change in the pattern of gene expression and the elevated synthesis of a family of heat shock or stress-induced proteins (*1*, *2*). Heat shock proteins ensure survival under stressful conditions that, if left unchecked, would lead to irreversible cell damage and ultimately to cell death. Heat shock proteins have essential roles in the synthesis, transport, and folding of proteins and are often referred to as molecular chaperones (*3*).

For molecular biologists, the heat shock response represents a beautiful example of inducible gene expression. In eukaryotic cells, this response involves transcriptional activation mediated by a transcription factor known as heat shock factor (HSF). In unstressed cells, HSF is present in both the cytoplasm and nucleus in a monomeric form that has no DNA binding activity. In response to heat shock and other physiological stresses, HSF assembles into a trimer and accumulates within the nucleus. The response to heat shock is rapid; activation and binding of HSF to the heat shock element (HSE), a specific DNA recognition sequence located in the 5'-flanking sequences of heat shock–responsive genes, are detected within minutes of temperature elevation (*4*–*7*). Although the kinetics and magnitude of DNA binding activity during heat shock are often proportional to the transcriptional response, HSF DNA binding activity does not always correlate with transcriptional activity, suggesting that there are multiple steps in the activation process (*8*, *9*). In addition, HSF exhibits a stress-dependent phosphorylation that may modulate its transcriptional activity (*7*, *10*). The heat shock transcriptional response attenuates upon prolonged exposure of cells at intermediate heat shock temperature (42°C) or upon return to physiological temperature (37°C); this attenuation is accompanied by conversion of the active trimeric form of HSF to the non-DNA binding monomer and by a return to the normal subcellular distribution. In contrast, prolonged exposure to extreme temperature (43°C) results in sustained heat shock gene transcription and HSF DNA binding activity.

The stress-dependent conversion of HSF to its active DNA binding form implies that HSF is negatively regulated. This regulation is not an intrinsic property of the protein as expression of recombinant *Drosophila*, chicken, mouse, and human HSFs in *Escherichia coli* yields a constitutively active DNA binding factor (*11*–*13*). Conceivably, the DNA binding ability of HSF in eukaryotic cells is controlled by a regulatory protein not present in *E. coli*. It has been speculated, on the basis of early studies with *Drosophila* and yeast, that heat shock proteins themselves may negatively regulate heat shock gene expression via an autoregulatory loop. According to this hypothesis, the increased levels of misfolded proteins induced during heat shock and other forms of stress sequester hsp70, resulting in the activation of HSF. Support for the autoregulatory hypothesis comes from the observation that the heat shock transcriptional response is correlated with increased levels of denatured and misfolded proteins (*14*). Likewise, activation of heat shock transcription in human cells by intermediate elevated temperatures is blocked by incubation with protein synthesis inhibitors, suggesting that misfolding or aggregation of nascent polypeptides may somehow trigger the response. Molecular chaperones such as members of the hsp70 family may be important in autoregulation of the heat shock response as they have been shown to facilitate protein folding by stabilizing intermediate folded states of nascent proteins, thus preventing them from engaging in inappropriate interactions that may lead to irreversible, nonspecific aggregation.

The results of in vitro experiments support a regulatory role for hsp70 in HSF activation. Inactive HSF in cytoplasmic extracts from non-heat-shocked HeLa cells can be converted to the DNA binding state by exposure to heat, non-ionic detergents, or low pH (*10*, *15*); however, addition of hsp70 blocks this conversion (*16*). The inhibitory effect of hsp70 on HSF activation is relieved by addition of adenosine triphosphate, an essential feature of heat shock protein function. These observations suggest that the inhibition is mediated by hsp70, possibly through alteration of the native conformation of HSF. Although a stable interaction



A model of HSF regulation. In the unstressed cell, HSF is maintained in a monomeric, non-DNA binding form through its interactions with hsp70 (1). Upon heat shock or other forms of stress, HSF assembles into a trimer (2), binds to specific sequence elements in heat shock gene promoters (3), and becomes phosphorylated (4). Transcriptional activation of the heat shock genes leads to increased levels of hsp70 and to formation of an HSF-hsp70 complex (5). Finally, HSF dissociates from the DNA and is eventually converted to non-DNA-binding monomers (6).

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between hsp70 with the inactive form of HSF has not yet been directly demonstrated, complexes containing hsp70 and the active trimeric form of HSF have been detected in extracts of heat-shocked cells (*16*). The association between the HSF trimer and hsp70 may be important in the conversion of the active HSF trimer to the monomer, a key event in the attenuation of the heat shock transcriptional response. The possible involvement of other heat shock proteins in HSF regulation has not yet been ruled out, however.

Many of the current observations are consistent with the following model for regulation of HSF (see figure). Under nonstressful conditions, HSF is maintained in a non-DNA binding form through transient interactions with hsp70 that perhaps stabilize a specific conformation of HSF. During heat shock, the appearance of misfolded or aggregated proteins creates a large pool of new protein substrates that compete with HSF for association with hsp70. Thus, heat shock and other stresses initiate the events that remove the negative regulatory influence on HSF DNA binding activity. The released HSF assembles into trimers and binds to DNA. Activation of HSF DNA binding leads to the elevated transcription, synthesis, and accumulation of heat shock proteins—in particular hsp70, which then associates with HSF. The association of HSF with hsp70 may be important in the regulation of its transcriptional activity or conversion back to the monomeric form.

The recent cloning of HSF genes in higher eukaryotes has revealed a family of HSFs containing at least three members, HSFs 1, 2, and 3 (*12, 13, 17, 18*). All HSFs share several structural features: a DNA binding domain at the NH_2-terminus, an adjacent cluster of hydrophobic amino acids organized into heptad repeats (leucine zippers), and a distally located heptad repeat near the COOH-terminus. HSFs 1, 2, and 3 are simultaneously expressed in most but not all cells, and the DNA binding activity of each is negatively regulated (*13*). Deletion of the COOH-terminal heptad repeat or introduction of nonconservative amino acid substitutions into this repeat result in constitutive DNA binding activity, thus implicating this leucine zipper in negative regulation (*13, 19*).

The existence of a family of HSFs offers an explanation for the observation that heat shock gene expression is induced during specific stages of development and differentiation. Experiments with antibodies specific for each HSF hint that there are functional differences between family members. HSF1, but not HSF2, becomes activated in response to elevated temperatures, heavy metals, amino acid analogs, and oxidative stress, whereas HSF2, but not HSF1, is activated during hemin-induced differentiation of erythroleukemia cells and is abundantly expressed during mouse spermatogenesis (*7, 14, 20*). Thus, the signals that activate the DNA binding properties of each factor are specific. How these signals result in the differential activation of the HSFs remains a puzzle. Interestingly, HSF3 does not become activated by conditions that affect HSF1 or HSF2, raising the possibility that there may be other undiscovered cellular "stresses" (*13*).

Abnormal expression of stress proteins has been widely observed in a number of disease states, including oxidant injury, ischemia, cardiac hypertrophy, fever, inflammation, metabolic diseases, tissue trauma, neuronal injury, and cancer, as well as in experimental models for aging (*1*). Thus, physiological "stress" may encompass an even broader array of conditions than previously considered. Do all cells respond to stress in an identical manner or are there a range of cell and tissue responses and a range of biologically interesting molecules that modulate heat shock response? Recently discovered examples of HSF inducers include salicylates, prostaglandins, and arachidonic acid, all of which are involved in the inflammatory response (*9*). Treatment of cells with any of these molecules at low, clinically relevant concentrations lowers the temperature at which the heat shock response is observed. Thus, as we learn more about the molecular mechanisms involved in HSF activation, it may become possible to identify new drugs that serve as agonists or antagonists of HSF activity. Finally, the expression of stress-induced genes may be useful in molecular toxicology and pharmacology studies as a marker of impending cell damage.

References and Notes

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