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MINI REVIEW

Surprising features of transcriptional regulation of heat shock genes

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The search for a common mechanism of heat shock gene activation has uncovered a surprising complexity of transcriptional signals that confer basal cell growth and developmental regulation, tissue-specific expression, heat shock, and other forms of stress-responsiveness. The myriad conditions that activate heat shock gene expression have puzzled many investigators since Ritossa (1962) demonstrated that heat shock induced a small number of Drosophila salivary gland chromosomal puffs. Two subsequent studies broadened the utility of this seminal observation. In 1974, Tissieres, Mitchell, and Tracy demonstrated that heat shock of Drosophila salivary glands induced the synthesis of a set of "heat shock" proteins; and in 1975, Spradling et al. and McKenzie et al. found that the response could be studied in cultured Drosophila cells. These observations inspired a plethora of studies on the regulation and function of the heat shock response, which is now recognized as a fundamental and universal reaction by which cells cope with the often adverse environmental circumstances in which they find themselves and which they are unable to control. This response has been observed in every species examined thus far, from bacteria to humans. Heat shock proteins, whose function is presumably to protect cells from the harmful physical and/or chemical effects of such stress, represent one of the most conserved families of proteins in evolution (reviewed in Morimoto et al., 1990).

Heat shock response has been the subject of numerous recent reviews (Pardue et al., 1989; Morimoto et al., 1990; Nover, 1990; Schlesinger et al., 1990; Sorger, 1991). In this minireview, we have limited our discussion to the transcriptional regulation of vertebrate heat shock genes with an emphasis on (1) the complexity of trans-acting factors and promoter elements that respond to a multitude of regulatory signals, (2) the response of vertebrate cells to stress by activation of heat shock transcription factor (HSF), and (3) the cloning and expression of multiple HSF genes.

The analysis of heat shock gene promoters reveals a surprising complexity of cis-acting elements

The typical view of the transcription regulatory regions of heat shock genes is that of a relatively simple promoter containing a TATA box and multiple inverted copies of the heat shock element (HSE) pentamer nGAAn that binds heat shock transcription factor (HSF). Although this description applies to many of the heat shock genes of S. cerevisiae and Drosophila, vertebrate heat shock genes are also cell-cycle regulated, serum-responsive, and induced by certain growth factors, mitogens, and viral infections (Wu and Morimoto, 1985; Wu et al., 1986; Milarski and Morimoto, 1986; Ferris et al., 1988; Ting et al., 1989; Phillips et al., 1991). For example, studies on the cis-acting elements of the human HSP70

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gene have identified a basal promoter, a clustered array of multiple copies of cis-acting promoter elements (CCAAT, Sp1, ATF, TFIID) that is necessary for growth-regulated expression and viral induction (Wu et al., 1986; Williams et al., 1989; Taylor et al., 1990). Many of these cis-elements are common to other housekeeping and growth-regulated genes. The basal elements are not essential for the stress-inducible transcriptional response, which is primarily dependent on the heat shock element, but they are necessary to attain maximal levels of heat shock responsiveness (Williams and Morimoto, 1990). Among vertebrate heat shock genes, the promoters are complex and may reflect the transcriptional machinery's sensitivity to the many physiological conditions affecting biosynthetic events in the cell, particularly protein metabolism.

The relationship between growth-regulated expression of HSP70 and the ability of certain DNA viruses to activate stress gene expression has been an intriguing problem. Only a subset of DNA viruses induces stress gene expression, and only certain stress genes are induced during viral infection (Phillips et al., 1991). Infection of primate cells by the DNA viruses adenovirus, herpes virus, and cytomegalovirus selectively induces transcription of the HSP70 gene, while infection with vaccinia virus and SV40 have no effect on stress gene expression (Kao and Nevins, 1983; Wu et al., 1986; Notarianni and Preston, 1982; Russell et al., 1987; Santomenna and Colberg-Poley, 1990; Phillips et al., 1991). Infection with the RNA viruses SV5, Sendai virus, Newcastle disease virus, and influenza virus induces only the transcription of the lumen-localized GRP78/BiP gene (Watowich et al., 1991). While there is some information on the mechanism by which certain viruses induce stress protein expression, what remains unclear is why stress proteins are induced. Some possibilities include an involvement in viral genome replication or in virion assembly.

During infection of primate cells with adenovirus, the synthesis of HSP70 is induced. This observation has long posed a dilemma, in that there is an apparent specificity of activation of cellular HSP70 gene transcription via the adenovirus E1a-13S protein (Wu et al., 1986; Phillips et al., 1991). This specificity is puzzling, since other members of the HSP70 gene family (p72/HSC70 and GRP78/BiP) and other heat shock genes (HSP90) share many of the same basal elements. Furthermore, there are hundreds—if not thousands—of other transcriptionally active cellular promoters which could provide potential targets for E1a; yet these other genes do not appear to be induced. It has been difficult to identify a specific target for viral or cellular gene trans-activation by E1a, as the respective target promoters share little in common. By using transient transfection assays and a collection of HSP70 promoter mutations, it has been shown that E1a trans-activates transcription through interactions with factors that compose the basal transcription complex of the HSP70 promoter (Williams et al., 1989; Taylor et al., 1990). Either the HSP70 basal promoter has a unique array of cis-acting elements, or the effect of E1a could be mediated through novel features of the chromatin structure of the endogenous HSP70 gene.

The heat shock response: simple in appearance but complex in details

The heat shock response represents one of the best characterized paradigms for understanding how the cellular transcriptional machinery senses and responds to fluctuations in the physiological environment of a cell. The induction of heat shock gene expression is mediated by the activity of heat shock transcription factor (HSF). HSF binds to arrays of inverted repeats of the pentamer nGAAn, which correspond to the heat shock elements (HSEs) in the promoters of heat shock genes and activate transcription of these genes (Xiao and Lis, 1988; Abravaya et al., 1991a; Mosser et al., 1988; Kingston et al., 1987). The DNA-binding and transcriptional activity of HSF are regulated differently in different species. In the yeast S. cerevisiae, HSF exists in non-stressed cells already bound to the HSEs of heat shock promoters, and it is a heat shock induced phosphorylation of HSF which makes it transcriptionally active (Sorger and Pelham, 1988; Jakobsen and Pelham, 1988; Sorger, 1990). In Drosophila and vertebrate cells, however, HSF is present at normal temperatures in a latent non-DNA binding form. Following heat shock or other forms of stress, HSF acquires DNA-binding ability and becomes transcriptionally competent (Zimarino and Wu, 1987; Kingston et al., 1987; Sorger et al., 1987). In yeast and heat shocked Drosophila cells, HSF exists as an oligomer (trimer or hexamer) in solution and when bound to DNA (Sorger and Nelson, 1989;

Perisic et al., 1989; Clos et al., 1990). The control form of HSF which pre-exists in a cryptic non-DNA binding state prior to heat shock appears to be a monomer (or dimer) which multimerizes to an apparent hexamer as the factor acquires sequence-specific DNA-binding ability and transcriptional activity (Abravaya et al., 1991b; K. Sarge and R. I. Morimoto, unpublished data). Thus, at least in eukaryotes, it appears that one level of HSF function is regulated by the oligomerization of the factor.

The mechanism of activation of HSF in eukaryotes appears to involve changes in protein conformation either in HSF itself or in other proteins which may act on HSF (Larson et al., 1988; Zimarino et al., 1990; Mosser et al., 1990). Heat shock induced HSF is phosphorylated, but it is unclear what role this modification plays in the transcriptional function of HSF (Sorger and Pelham, 1988; Larson et al., 1988). The molecular response to elevated temperatures is rapid; binding of HSF to DNA can be detected within a minute of heat shock (Abravaya et al., 1991c). The binding of HSF to DNA appears to be highly temperature-sensitive and is not dependent on new protein synthesis (Abravaya et al., 1991a,b,c; Zimarino and Wu, 1987). During recovery from heat shock (the attenuation phase), HSF dissociates from the HSE, presumably undergoing a conformational change, and returns to the cryptic, non-DNA binding form. It is tempting to speculate that the dissociation and conversion of HSF during the transition from an active to inactive state may involve interaction with a heat shock protein. Indeed, preliminary results indicate that HSF induced during heat shock and hemin treatment is associated with HSP70 (K. Abravaya, M. Myers, and R. Morimoto, unpublished observation).

The genes encoding HSF have been isolated from a number of species, including S. cerevisiae, K. lactis, Drosophila, tomato, chicken, mouse, and human (Wiederrecht et al., 1988; Sorger and Pelham, 1988; Jakobsen and Pelham, 1991; Clos et al., 1990; Scharf et al., 1990; Rabindran et al., 1991; Schuetz et al., 1991; Sarge et al., 1991; A. Nakai, personal communication). There appears to be only a single HSF gene in S. cerevisiae and K. lactis, and in Drosophila only one HSF gene has been isolated thus far. Thus, it was of some surprise that the genomes of tomato, chicken, mouse, and human contain multiple HSF genes. Much of the homology between HSFs of different species is located in the DNA-binding and oligomerization domains in the N-terminal half of the proteins. The conserved DNA-binding domain of HSF does not share homology with any known DNA-binding structural motif and appears to represent a new class of DNA-binding protein. The HSF oligomerization domains contain a highly conserved leucine zipper motif consisting of overlapping series of heptad repeats of hydrophobic amino acids (Sorger and Nelson, 1989; Clos et al., 1990). The presence of multiple heat shock factors in a single species raises many interesting questions. Are HSF multimers composed only of identical HSF subunits (homomultimers), or can they contain a mixture of different HSF subunits (heteromultimers)? If heteromultimers do form, regulation of the relative stoichiometry of HSF subunits could modulate the function of the HSF complex. This might provide various cells with the ability to finely tune the function of HSF to suit their individual needs. Yeast HSF contains two physically separable transcriptional activation domains that mediate distinct transient and sustained responses of HSF to stress stimuli (Sorger, 1990). Evidence that HSF in higher eukaryotes may also have transient and sustained response capabilities has been obtained from experiments in which HeLa cells heat shocked at 42°C show transient HSF activation that attenuates over the course of the heat shock, while at 43°C the HSF activity is induced and maintained at high levels even after four hours of continuous heat shock (Abravaya et al., 1991c).

Other possible roles for multiple HSF genes include the potential for differential regulation of heat shock gene inducible transcription. For example, each factor could respond to a different stress signal or set of signals. This could explain the seeming diversity of many of the myriad known inducers of the stress response (Morimoto et al., 1990). Another possibility is that one factor could act positively on transcription, and the other negatively. Alternatively, it is possible that some species have divided HSF function into separate inducible and constitutively active factors. The inducible activity could respond to stress signals and activate the classical heat shock response, while the constitutive activity could be used to turn on heat shock genes in the absence of stress, such as during specific developmental stages, or perhaps in

cells that require higher basal levels of heat shock proteins. This possibility is consistent with the observations of developmentally regulated expression of heat shock proteins during mouse embryogenesis and differentiation of the mouse male germ line and the high levels of constitutive HSE-binding activity in unstressed embryonal carcinoma cells (Barnier et al., 1987; Zakeri and Wolgemuth, 1987; Mezger et al., 1989).

Comparisons of the heat shock transcriptional response in S. cerevisiae, Drosophila, and vertebrate cells: the role of basal elements in the inducible response

In the promoters of the Drosophila and vertebrate heat shock genes, the TATA binding factor, TFIID, is bound prior to heat shock; HSF, induced upon heat shock, binds to the HSEs, resulting in transcriptional activation (Wu, 1984; Thomas and Elgin, 1988; Abravaya et al., 1991a). Evidence from both Kingston's and Wu's laboratories on the relationship between HSF and nucleosomes reveals that HSF cannot displace nucleosomes in vitro (Taylor et al., 1991; Becker et al., 1991). Since the heat shock promoter is in an open chromatin nucleosome-free state in vivo (Wu, 1984), it would be necessary for another factor, perhaps a component of the basal transcription complex in the vertebrate heat shock gene promoters, to displace nucleosomes. Studies on the Drosophila HSP26 promoter have identified a CT/GA-factor that may be involved (Gilmour et al., 1989; Glaser and Lis, 1991), while in vertebrate cells Sp1 could have a similar role, since this factor appears to be tightly associated in vivo with the Sp1 site in the HSP70 basal promoter under conditions where basal transcription is low (Abravaya et al., 1991a; B. Phillips, K. Abravaya, and R. Morimoto, unpublished data). Another potential distinction is the involvement of a poised polymerase complex that regulates the transcription of the Drosophila heat shock genes (Rougvie and Lis, 1988); however, there is no evidence to rule out the possibility that a poised polymerase complex could also regulate the vertebrate heat shock response.

In S. cerevisiae, HSF is constitutively bound to the HSE prior to heat shock, whereas in other eukaryotes HSF does not bind to DNA until it is activated by heat shock and other forms of stress. This distinction between DNA-binding properties of yeast and other eukaryotic HSFs, it has been suggested, represents a fundamental difference in the properties of HSF. The lack of intermediates in this reaction under conditions of heat shock has supported the expectation that activation of HSF in mammalian cells involves the concomitant conversion of a non-DNA binding control form of HSF to a DNA-bound transcriptionally active oligomeric state. However, recent studies on salicylates, one of the original compounds shown to induce Drosophila heat shock puffs (Ritossa, 1963), reveal that saliclyate treatment of human cells uncouples the activation of the heat shock response. Low levels of salicylate induce HSF, which binds in vivo to the HSEs of the HSP70 promoter yet does not activate transcription of the HSP70 gene (D. Jurivich, L. Sistonen, and R. Morimoto, in preparation). These results indicate that activation of the DNA-binding domain of HSF can be uncoupled from transcriptional competence and suggest that activation of HSF is a multi-step event.

The availability of cloned HSF genes will facilitate not only the elucidation of the biochemical steps in HSF activation and the role of heat shock proteins in regulating HSF activity, but also the discovery of other cellular factors that influence DNA binding and transcription.

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