Gene Expression, Vol. 7, pp. 271–282, 1999
Printed in the USA.

1052-2166/99 $10.00 + .00

Heat Shock Factor Function and Regulation in Response to Cellular Stress, Growth, and Differentiation Signals

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Heat shock factors (HSF) activate the transcription of genes encoding products required for protein folding, processing, targeting, degradation, and function. Although HSFs have been extensively studied with respect to their role in thermotolerance and the activation of gene expression in response to environmental stress, the involvement of HSFs in response to stresses associated with cell growth and differentiation, and in response to normal physiological processes is becoming increasingly clear. In this work, we review recent advances toward understanding how cells transmit growth control and developmental signals, and interdigitate cellular physiology, to regulate HSF function.

Hsp HSF Heat shock Cell cycle Stress Growth signals

ORGANISMS and cells are constantly exposed to a diverse array of stresses to which they must adapt. On one hand, these stresses include a vast number of environmental stimuli such as elevated temperature, exposure to toxic organic and inorganic chemicals, the presence of invading pathogens, and other environmental insults. On the other hand, many stresses that pose hardships to all organisms are a consequence of normal growth, cell duplication, metabolism, and differentiation. When faced with stressful and or potentially toxic conditions, it is critical that cells have the capacity to sense, evaluate, and respond appropriately to adapt to their constantly changing intra- and extracellular environments. It is well established that cells which continue to progress through the cell cycle in the presence of genotoxic substances accumulate genetic damage that has the potential to lead to the transformed state. Fortunately, cells utilize proteins such as p53 to suspend growth until DNA damage has been repaired, or until cells are equipped with an array of gene products that allow cell cycle progression to occur, even in the continued presence of stress (46). Therefore, sensing and responding to DNA damage, or other defects in key cellular regulatory events that lead to stress-induced disruption of the cell cycle, is critical for survival. Indeed, this would include the temporary cessation of cell cycle progression when rapidly proliferating cells initiate one or more programs of differentiation.

Eukaryotic organisms harbor heat shock transcription factors (HSFs), a structurally and functionally conserved class of proteins that respond to a diverse array of cellular and environmental signals to regulate gene expression and that play crucial roles in the adaptation to stress. As shown diagramatically in Fig. 1, the fundamental anatomy of HSF molecules is conserved from yeast cells to humans, with a DNA binding domain related to the prokaryotic helix–turn–helix motif and two sets of coiled-coil regions, thought to be involved in both intramolecular interactions that sequester the proteins as inactive monomers, and intermolecular interactions to form homotrimeric molecules capable of binding to the cognate *cis*-acting DNA element (HSE, heat shock element) with high affinity. Most HSF molecules are known to possess a carboxyl-terminal *trans*-activation domain and other, internal repression regions that regulate the use of these *trans*-activation domains [see (86) for a detailed review]. HSF from the baker's yeast, *Saccharomyces cerevisiae*, however, contains both an amino-terminal activation domain (NTA) and a carboxyl-terminal *trans*-activation domain (CTA),

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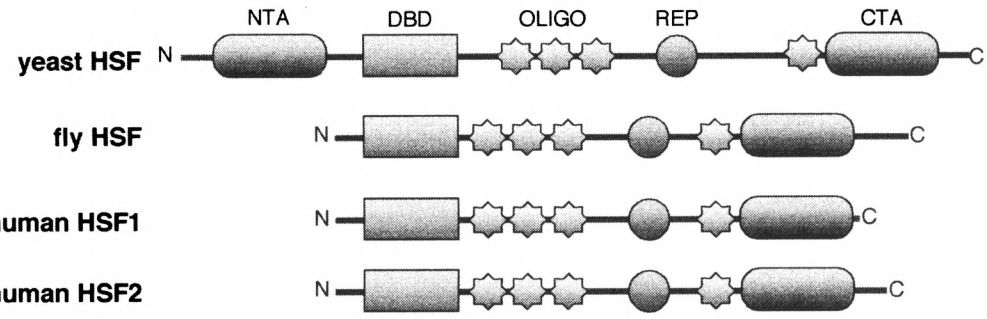

FIG. 1. Conserved domain architecture of heat shock transcription factors. Critical regions of HSF are depicted with approximate relative locations in the different HSF molecules. Graphic representations are as follows: *rounded rectangle*, transcriptional activation domain (NTA, CTA); *rectangle*, DNA binding domain (DBD); *star*, leucine zipper motif oligomerization domain (OLIGO); *circle*, repression domain (REP). For simplicity, only HSF1 and HSF2 are shown from humans.

which play distinct roles in the stress response in this organism (49,75,79). Although HSF proteins have been extensively investigated with respect to their role in the activation of genes encoding proteins that protect cells from external stressful conditions, it has only recently become clear that HSFs play key roles in sensing growth control and differentiation signals and in the regulation of cell cycle progression. In this review, we focus on recent advances in the regulation of HSF by both environmentally induced and cell growth- and differentiation-induced stresses.

REGULATION OF HSF BY GROWTH CONTROL SIGNALS

In mammalian HSF1, a conserved central repression domain, localized between amino acids 221 and 313, is known to modulate repression of the HSF1 *trans*-activation domain under normal growth temperatures, as well as to confer heat shock inducibility during stress (43,44). Recently, a number of reports have suggested that the central repression domain responds to cellular growth control signals to regulate the function of the carboxyl-terminal *trans*-activation domain at normal growth temperatures, as depicted in Fig. 2. Consistent with the observation that the repression domain is composed of 24% serine residues, the hypothesis that phosphorylation of this domain plays a central role in its activity was borne out by both in vivo and in vitro phosphorylation studies. Indeed, it has been demonstrated that in vitro phosphorylation at a critical serine residue at amino acid position 303 (S303) is primed by prior phosphorylation at serine 307 (S307) by a mitogen-activated protein kinase (MAPK) (15). A key regulatory phosphorylation at S303 appears to be carried out by glycogen synthase kinase 3 (GSK-3), because this enzyme will phosphorylate S303 in vitro, provided that the priming phosphorylation of S307 has occurred, and overexpression of GSK-3 by transfection inhibits HSF1-dependent control of *hsp70* basal expression levels (16,87). Consistent with these observations, it has been demonstrated that mutation of the S303 or S307 residues to alanine, either in the context of a Gal4 DNA binding domain–HSF1 *trans*-activation domain fusion, or in full-length HSF1, gives rise to deregulated *trans*-activation activity under non-heat shock conditions (43). Conversely, mutation of S303 and S307 to glutamic acid, mimicking the constitutively phosphorylated state, resulted in properly regulated HSF1 activity, suggesting that dephosphorylation of these residues may not be required for activation (43).

Recently, it has been shown that stimulation of the Raf/Erk MAP kinase pathway, normally responsive to a number of cellular growth factors, stimulates increased phosphorylation of the proline-directed S303 and S307 residues (33). Furthermore, overexpression of a dominant negative allele of Erk1, but not Erk2, increased *hsp70* reporter gene activity, suggesting the specific involvement of the Erk1 isoform in negatively regulating HSF1 (57). Although often of limited specificity, the administration of sodium vanadate, an inhibitor of MAP kinase phosphatase, resulted in increased phosphorylation of HSF1, delayed activation of the *hsp70* target promoter, and caused a reduction in induced thermotolerance (57). Taken together, these studies strongly suggest that cellular kinases intimately involved in sensing growth signals play a key role in negatively regulating the basal levels of HSF1 *trans*-activation activity under "normal" growth conditions. Furthermore, these re-

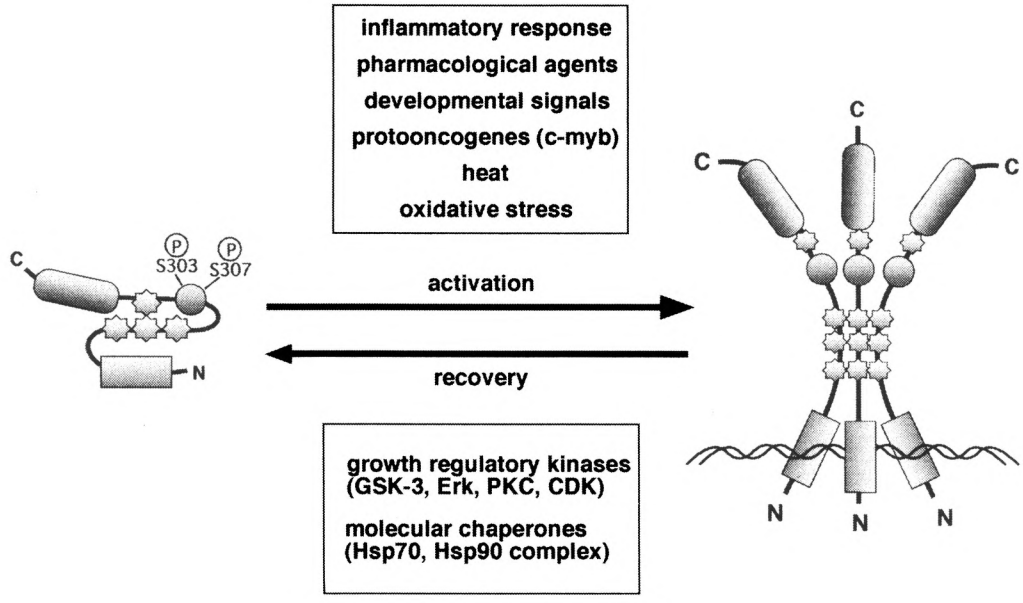

FIG. 2. Cellular regulation of HSF. The inactive, monomeric form (left), and the transcriptionally active, trimerized HSF (right), are depicted along with signals and protein effectors responsible for regulation (see text for details). Serine residues that lie within the repression domain at positions 303 and 307, as numbered in human HSF, are depicted in their phosphorylated state in the inactive HSF molecule to reflect the negative role of phosphorylation in HSF regulation. Leucine zipper 4, found immediately proximal to the transcriptional activation region, is shown engaged in an intramolecular bridge thought to restrain HSF activity.

sults imply that HSF1 activity is responsive to cellular growth signals or physiological stresses that occur as a function of these responses. In contrast to the role protein phosphorylation may play in modulating the activity of the HSF1 *trans*-activation domain, it has also been recently reported that an *Arabidopsis* cyclin-dependent protein kinase phosphorylates one of the plant HSF isoforms, AtHSF1, resulting in inhibition of sequence-specific binding to cognate HSEs (67). Therefore, protein kinases intimately linked to cell cycle progression and cellular growth responses may influence the activity of HSF proteins at the level of either DNA binding or *trans*-activation.

Why might it be important to regulate HSF1 basal activity? A number of studies suggest that HSF1 participates in both negative and positive events to regulate the expression of both well-characterized heat shock proteins (Hsps) and important cellular growth control proteins. Studies in *Drosophila* have demonstrated that overexpression of Hsp70 at normal growth temperatures is detrimental to cell growth (28). Furthermore, it has been demonstrated that HSF1 negatively regulates the expression of the pro-interleukin 1β gene, via a *cis*-acting HSE in the IL-1β promoter, under both normal and heat shock conditions (10,11). Although the precise mechanism for this HSF-mediated inhibition is not known, it has been suggested that this form of regulation may serve to temper the inflammatory response by rendering cytokine gene expression transient (11). In addition to its negative regulatory role in IL-1β expression, evidence suggests that HSF1 also negatively regulates Ras-induced c-*fos* gene expression (14). Interestingly, this negative regulation requires neither the HSF1 DNA binding nor *trans*-activation function, suggesting that it occurs via a distinct mechanism to that observed on the IL-1β promoter.

The observation that the *hsp70* gene is potently induced in mammalian cells at the G_1/S phase transition of the cell cycle strongly suggests that Hsp70 plays an important role in cell cycle progression (39,56). Although it is unclear what triggers Hsp70 expression during the cell cycle, recent reports link the c-Myb protein to HSF activation. First, it was demonstrated that expression of c-Myb activates *hsp70* gene expression, in a manner dependent on the *hsp70* promoter HSE, in CV-1 cells but not in NIH 3T3 cells (38). Recently, it has been shown that, although c-Myb does not directly interact with HSEs, c-Myb does stimulate transcription from an HSE reporter gene when cotransfected into NIH 3T3 cells in concert with the chicken HSF3 cDNA (37). The observations that activation occurs in unstressed cultured cells, that c-Myb forms a complex with chicken HSF3 through their respective DNA binding domains, and that this association stimulates HSF3 nuclear entry all suggest that c-Myb modulates the activity of HSF3 in normal proliferating cells (37).

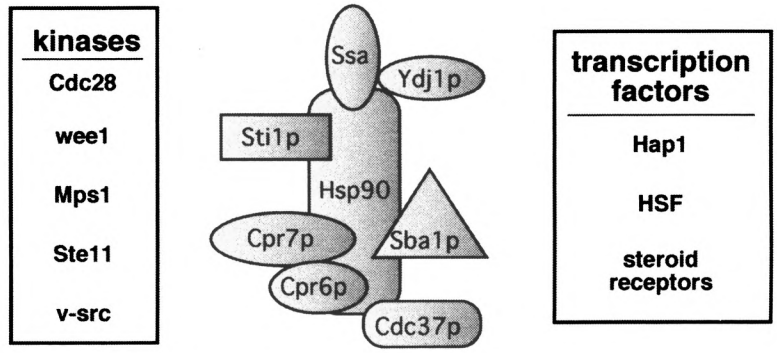

FIG. 3. The Hsp90 chaperone complex in yeast. The subunits of the Hsp90 chaperone complex as found in yeast are depicted together, although it should be noted that composition of the complex is dynamic and involves the sequential binding and release of these co-chaperones. Two classes of protein have been shown to require the Hsp90 complex to function, protein kinases and transcription factors, and representatives of these classes with experimental evidence for interaction from the yeast system are shown [Cdc28 (31); wee1 (3); Mps1 (70); Ste11 (50); v-src (88); Hap1 (91); HSF (24, 60); steroid receptors (63)].

Consistent with this notion, c-Myb is highly expressed in the G_1/S transition and is required for the maintenance of the proliferative state (32). It should be noted that, although c-Myb could not *trans*-activate *hsp70* gene expression in concert with coexpressed mammalian HSF1 or HSF2, it is possible that an as yet unidentified functional homolog to the chicken HSF3 may exist in mammalian cells.

REGULATION OF HSF BY COMPONENTS OF THE Hsp90 CHAPERONE COMPLEX

A critical role that HSF molecules play in all eukaryotic cells is to activate both basal and stress-induced transcription of genes encoding components of the Hsp90 chaperone complex, which is essential for the folding, and maintenance in an activation-competent state, of a large number of key cellular regulatory proteins. To date, experimentally demonstrated substrate proteins include steroid hormone receptors [e.g., glucocorticoid (GR); progesterone (PR)], the Raf and v-src protein kinases, the tumor suppressor protein p53, endothelial nitric oxide synthase, cell cycle regulatory protein kinases, and several transcription factors [for a detailed review, see (64)]. This chaperone complex is remarkably conserved from yeast to humans in terms of its function on substrate proteins and its repertoire of functional subunits (12). In yeast, the Hsp90 chaperone complex has been demonstrated to contain Hsp90, Hsp70, Ydj1 (42), the cyclophilin orthologs Cpr6 and Cpr7 (23), the p60 ortholog Sti1 (13), Cdc37 (41), and the p23 ortholog Sba1 (9,27), with perhaps other subunits, both functional and regulatory, yet to be identified (Fig. 3).

A key genetic experiment in yeast cells first suggested that the products of HSF target genes, contained within the Hsp90 chaperone complex, serve to negatively regulate the activity of HSF. When a number of yeast genes encoding members of the SSA family of Hsp70 proteins were deleted, cells were shown to have elevated levels of other Hsps, at least some of which were known to be under the control of HSF, under nonstress conditions (61). Subsequently, transfection experiments in mammalian cells suggested that Hsp70 is important, though not sufficient, to negatively regulate HSF activity (1). Recent biochemical experiments support this idea. Some of these experiments demonstrated that high-level constitutive expression of Hsp70 both reduces the extent of HSF activation in response to heat shock and facilitates the recovery from heat shock as ascertained by the inactivation of HSF DNA binding (40). Additional experiments, based upon coimmunoprecipitation assays, demonstrated that Hsp70 interacts with HSF both in the inactive and DNA binding active form, and that, unlike other Hsp70–substrate interactions, the Hsp70–HSF interaction was not significantly disrupted in the presence of ATP (65). Although this study could find no accelerated inactivation of HSF DNA binding activity by high-level expression of Hsp70 alone, conditions under which the entire repertoire of heat shock proteins are produced did result in early inactivation of HSF DNA binding activity after prolonged heat shock (6). Consistent with observable Hsp70 chaperone activity in mammalian cell nuclei, recent studies suggest that Hsp70, as well as the co-chaperone Hdj1, directly interact with the HSF1 *trans*-activation domain, thereby inhibiting the activity of HSF1 once bound to DNA (55,72). It is also interesting that, given the highly inducible phosphorylation of HSF1 in response to stress, Hsp70 has been recently demon-

strated to inhibit that activation of stress-activated protein kinases including Jnk and p38 (29).

Two recent reports, using an in vitro HSF activation system or *Xenopus* oocytes, suggest that another component of the protein chaperone complex, Hsp90, associates with HSF1 to negatively regulate its activity. Both reports suggest that either depletion of Hsp90 or neutralization with Hsp90-specific antiserum activate the monomer to trimer transition for HSF1 (2,92). Indeed, it appears that depletion of other proteins found in the mammalian Hsp90 chaperone complex, including Hsp/c70, p23, CyP-40, Hip, or Hop, do not activate HSF1 trimerization, indicating that either free Hsp90 negatively regulates HSF1 or that the most important contacts between HSF1 and the Hsp90 chaperone complex are with Hsp90 itself (92). These studies together suggest a model in which, in unstressed cells, the inactive HSF1 monomer associates with Hsp90, and in response to stress, HSF1 oligomerization is accompanied by loss of Hsp90 from the complex. The reported roles of mammalian Hsp90 on HSF1 activation are consistent with recent observations in yeast, which demonstrated that nonfunctional forms of Hsp90, as well as deletions of the Cpr7 subunit of the Hsp90 chaperone complex, give rise to constitutive activation of HSF-responsive genes (24). Indeed, it is fitting that, like in prokaryotic systems, some of the products of genes activated by HSF1 serve to negatively regulate HSF1 function, because this is a convenient manner to fine tune and appropriately downregulate activated HSF1 (19).

HSF AND CELL PROLIFERATION

In the following sections, new aspects of HSF function will be investigated, starting with a review of recent data implicating HSF in cell growth and proliferation in both stressed and unstressed cells. Research in a number of experimental systems has uncovered new developmental roles for HSF and Hsps. Recent progress in understanding the requirements for HSF and Hsps in gametogenesis will be presented, followed by new findings tying HSF function to embryogenesis and cellular differentiation. Finally, we will conclude with an examination of HSF roles in two important aspects of cell biology with biomedical relevance: cancer and apoptosis.

The roles of HSF in providing cellular protection against environmental stress are well documented. This pivotal requirement for HSF is manifest in many cell types as a loss of acquired thermotolerance, defined as the ability to mount a protective response against severe heat shock after exposure to a mild stress, when HSF function is compromised. In addition to its role in stress response, however, HSF is also required for normal, unstressed growth in the single-celled yeasts *S. cerevisiae* and *Schizosaccharomyces pombe* (30,76,85). Little is known about this aspect of HSF biology, although the requirement is likely to reflect a need for HSF in basal transcription of a variety of target genes. For example, it has been demonstrated that removal of HSEs from the promoter region of the *HSC82* gene, encoding one of the yeast Hsp90 isoforms in *S. cerevisiae*, drastically reduces steady-state transcript levels in the absence of stress (52). The contribution of HSF to basal gene transcription must be modulated, however, as overexpression of HSF in yeast is toxic, as recently demonstrated in a genetic study in which the *HSF1* gene was isolated as a cDNA whose conditional overexpression blocked cell growth during the G_1/S transition phase of the cell cycle (26). The requirements for HSF function during normal cellular proliferation in higher eukaryotes have been difficult to discern, due to the multiple HSF isoforms expressed in these cells. However, the stimulation of *hsp70* expression in mammalian cells by c-Myb demonstrates that the stress response system governed by HSF can be mobilized by the cell proliferation machinery during normal cell cycle progression (37).

Genetic analysis of HSF mutations in budding yeast has provided mounting evidence for a requirement for an intact heat shock response for cell cycle progression during periods of prolonged growth under stress conditions. Such a function was first hinted at with the isolation of the temperature-sensitive HSF mutant *mas3* (74). This *HSF1* allele contains an amber mutation located in the portion of the gene encoding the trimerization domain, which is overcome by nonsense suppression, resulting in a stable but functionally thermolabile molecule (81). Upon shift to the restrictive growth temperature of 37°C, *mas3* mutant cells exhibit reversible arrest in the G_2/M phase of the cell cycle (74). A nearly identical phenotype was obtained with a truncated form of HSF termed HSF(1-583), which lacks the carboxy-terminal *trans*-activation domain (58). Cells expressing this mutant allele as their sole source of HSF show no obvious growth defects at normal growth temperatures, but cease growing after approximately 6 h at 37°C with a distinctive large-budded morphology, as illustrated in Fig. 4. Heat shock-arrested HSF(1-583) cells also contain a single replicated but undivided nucleus, consistent with G_2/M phase arrest. Genetic suppressor and protein expression analyses together determined that this mutated HSF is severely defective in both basal and heat shock-induced expression of the two genes encoding Hsp90 in yeast, *HSC82* and *HSP82*. Another allele of HSF1, specifi-

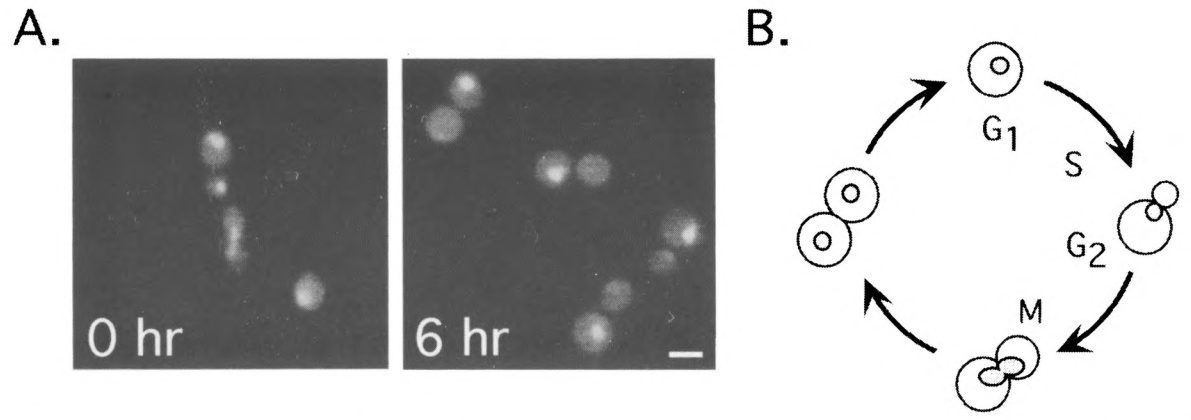

FIG. 4. Cell cycle arrest of an HSF truncation mutant (A) HSF(1-583) cells arrest in a late stage of the cell cycle. HSF(1-583) cells grown at 30°C or shifted to the nonpermissive growth temperature of 37°C for 6 h were stained with DAPI to visualize the nucleus with epifluorescence microscopy (bar, 10 μm). Note the large daughter buds in cells grown at 37°C relative to those at the preshift condition. In addition, the nucleus has failed to migrate to the bud neck in these cells for initiation of mitotic separation. (B) The cell cycle in yeast.

cally defective in Hsp90 production, was independently isolated in a screen for genes displaying synthetic lethality with a mutant allele of the primary cyclin-dependent kinase in yeast, *CDC28* (90). Extensive phenotypic characterization of arrested cells in this mutant highlighted severe defects in spindle pole body morphogenesis, suggesting a role for Hsp90 in this early phase of mitosis, which is yet to be fully elucidated. Roles for Hsp90 in growth may not be restricted to this phase of the cell cycle, however, as the molecular chaperone was also isolated in a screen for genes capable of overcoming G_1 arrest caused by hyperactivation of the pheromone response MAPK pathway in yeast (25).

The involvement of molecular chaperones in cell cycle progression is not limited to Hsp90 alone. Ample biochemical and genetic evidence implicates many of the known components of the Hsp90 chaperone complex in the function of cell cycle regulatory protein kinases, as illustrated in Fig. 3. For example, the Cdc37 protein physically interacts with the cyclin-dependent kinase Cdk4 in mammalian cells, and is required for full function of Cdc28p in yeast (31,77). The Hsp70 co-chaperone Ydj1 (Hsp40) also exhibits genetic interactions with the *CDC28* gene, and is absolutely required for function of the transforming kinase v-src, because loss of Ydj1p function will alleviate the toxicity caused by v-src overexpression in yeast (20,42,90). In keeping with the concerted functions of the Hsp90-associated co-chaperones, the synthesis of many of these proteins has recently been found to be coordinately regulated by HSF under both normal and stress conditions in yeast (X. D. Liu, K. Morano, and D. Thiele, manuscript in preparation).

In addition to its well-defined roles in steroid hormone signaling in mammalian cells, Hsp90 itself has been shown to be required for maturation and proper function of a number of signal transduction and regulatory kinases, including Raf (82), src (88), and the product of the *wee1*$^+$ gene in *S. pombe*, which negatively regulates the G_2/M transition (3). In many cases, the specific genetic or biochemical interactions detected with a distinct component of the Hsp90 chaperone system have led to the finding that the substrate of interest exists in a stable complex with multiple co-chaperone subunits. This was recently demonstrated for the cell cycle regulatory transcription factor p53, mutations in which correlate with tumor-promoting activity (8,71,84). It was found that mutated p53 molecules from a panel of breast cancer cell lines, but not wild-type p53, exist in stable complexes with Hsp90, Hsp70, CyP-40, and p23. Association of the chaperones with p53 blocks proteolytic turnover of this normally short-lived protein, resulting in dramatically increased levels of dysfunctional molecules, which may contribute to their transforming ability (84). The involvement of Hsps in human cancer will be further elaborated on below.

Clearly, the involvement of HSF and Hsps in cell growth and proliferation is a rapidly expanding facet of the stress response system. Given the central role of HSF in coordinating this response at the transcriptional level, it is of great interest to identify additional genes controlled by HSF under "normal" growth conditions and determine their contributions to the regulation of cell growth. Additionally, the participation of Hsps in modulating the function of key cellular regulatory molecules in normal or aberrant conditions is an exciting area for future study and may present a promising avenue for therapeutic intervention. For example, the National Cancer Institute

has selected the Hsp90 binding compound geldanamycin for clinical trials, based in part on its demonstrated ability to revert the growth of v-src-transformed cells. Indeed, treatment of cancer cells with the above-mentioned mutant forms of p53 with geldanamycin results in disruption of the p53–Hsp90 interaction and restores normal protein levels of the tumor suppressor (84).

ROLE OF HSF AND Hsps IN DEVELOPMENT: GAMETOGENESIS

An exciting new facet of Hsp gene regulation by HSF is the recent discovery that HSF also responds to developmental cues, coordinating the synthesis of Hsps and perhaps other as yet unknown genes at specific stages in a differentiation program. Recently, two independent lines of research into gene regulation during spermatogenesis have converged. It has long been known that a number of unique gene products are synthesized during spermatogenesis, among them members of the Hsp70 class of molecular chaperones. This makes intuitive sense, as it is well known that male germ cells are particularly sensitive to heat stress, relative to somatic cells. Germ cell-specific Hsp70 is encoded by two genes, *Hsp70-2*, whose expression is restricted to the meiotic phase of spermatogenesis (5,89), and *HSC70t*, expressed only in the postmeiotic phase (51). Both proteins are abundantly expressed during their respective developmental phases, and Hsp70-2 is associated with synaptonemal complexes in early meiosis, implying a substantive role for Hsp70 in spermatogenesis (4). This hypothesis was borne out by analysis of *Hsp70-2* knockout mice, which lack postmeiotic spermatids and mature sperm, and are therefore sterile (21). Interestingly, female mice homozygous for the mutant allele are fertile, consistent with the demonstration that the synaptonemal complex in pachytene oocytes lacks Hsp70-2 (4). How is developmental activation of Hsp70-2 achieved? Mammalian HSF2 has not been found to respond to heat stress in mammalian cells, and appears to be activated instead by developmental signals. Sarge and coworkers determined that the expression of HSF2 in mouse testis is itself developmentally regulated, and that HSF2 is activated for DNA binding (68). Furthermore, activated HSF2 binds the *Hsp70-2* promoter, as demonstrated by electrophoretic mobility shift assays using testis protein extract and different DNA fragements from the promoter region (22,68). Although these results are tantalizing, the precise mechanisms of HSF2 activation, and the factors controlling the hierarchical developmentally regulated expression of HSF2 and *Hsp70-2*, remain to be determined.

A requirement for HSF has also been observed during oogenesis in *Drosophila*. Four independent lethal mutations in the *hsf* gene were isolated in a study to probe the function of HSF in this animal system (36). When introduced as germ-line clones, three of the mutations resulted in female sterility, manifest as arrest in egg-chamber development. Interestingly, the requirement for HSF may not reflect a need for classical Hsp targets, as an *hsp83-lacZ* transgene displayed normal developmental induction in the hsf^1 mutant. Moreover, the developmental requirements for HSF function appear to be separable: one of the mutations isolated, hsf^4, is a temperature-sensitive allele that shows no defects in oogenesis upon inactivation but arrests growth early in larval development. Together, these findings substantially augment our understanding of HSF at the organismal level and imply a conserved role for HSF function in germ cell development in higher eukaryotes. In support of this proposal, it was found that over 150 sites, including developmental loci, accumulated HSF after heat shock in chromosomal spreads from *Drosophila* salivary gland, a number far in excess of known Hsp genes (83).

ROLE OF HSF AND Hsps IN DEVELOPMENT: EMBRYOGENESIS AND DIFFERENTIATION

The participation of HSF in mammalian development is not restricted to gametogenesis. The abundance of Hsps during certain periods of embryonic development in the absence of external stress prompted Mezger and colleagues to look for HSF activity during mouse embryogenesis (54). A constitutive DNA binding activity was indeed found to exist between the eight-cell and blastocyst stages. Supershift assays with HSF1- and HSF2-specific polyclonal antibodies demonstrated that the DNA binding activity was due almost exclusively to HSF2, consistent with reports of constitutive HSF2 DNA binding activity in embryonal carcinoma cells (59). Subsequent analysis determined that HSF2 expression and function was regulated both temporally and with tissue specificity: embryo-wide expression was highest between approximately 8 and 15 days postimplantation, after which it was restricted to the developing central nervous system, as demonstrated by immunoblot and in situ hybridization analyses (66). Surprisingly, peak HSF2 function did not correlate with expression of a number of developmentally regulated Hsps, again strengthening the notion that the function of HSF during development extends beyond induction of classical Hsps. A corollary to this conjecture is that developmental regulation of many of the in-

ducible Hsps must occur through other as yet unidentified transcription factors.

The elucidation of developmental HSF activity in both fly and vertebrates suggests a common dependence upon HSF function. Interestingly, in *Drosophila*, multiple roles are fulfilled by a single HSF, whereas HSF function in mammalian development has been thus far only been demonstrated for HSF2. What else is known about this intriguing HSF isoform? HSF2 was initially found to be responsive to hemin treatment in differentiating K562 erythroid cells (73). Cellular HSF2 is actually represented by two distinct HSF molecules produced by alternative splicing: HSF2-α and HSF2-β (69). HSF2-β lacks 18 residues located near the carboxyl-terminus, and is a less potent transcriptional activator when expressed in NIH 3T3 cells (69). However, the functional consequence of having two isoforms coexpressed is not clear. Leppa and coworkers recently demonstrated that the ratio of HSF2-α to HSF2-β may itself comprise a level of regulation, because overexpression of HSF2-β relative to HSF2-α resulted in repression of hemin-mediated transcriptional activation by HSF2 (45). Cells overexpressing HSF2-β were also defective in globin accumulation, a marker for erythroid differentation, suggesting that precise control of HSF2 activity in these cells is critical for proper development.

HSF IN CANCER AND APOPTOSIS

The work discussed above supports the involvement of HSF and Hsp function in a number of aspects of cellular proliferation, from cell cycle progression to germ cell development and differentiation. Based on these findings, it is not surprising then that accumulating evidence correlates HSF function and Hsp levels with two aspects of cell biology with profound biomedical significance: cancer and apoptosis.

The involvement of heat shock proteins in cancer biology is best studied in breast cancer. Overexpression of Hsp27 in breast carcinomas is associated with shorter disease-free survival, and levels of Hsp90 were also found to be substantially higher in malignant compared to benign breast tissue (35,80). Consistent with the high constitutive expression of Hsps, autoantibodies recognizing Hsp27 and Hsp90 are detectable in patients with metastatic cancer but not in healthy individuals (17,18). Moreover, the immune response supports nonidentical roles for different Hsps in cancer progression, as the presence of serum antibodies against Hsp90 is associated with decreased survival (18), whereas high levels of antibodies directed against Hsp27 correlates with improved survival (17). Although the precise functions of Hsps in cancer cell biology are still unknown, it is likely that something resembling a general stress response is evoked in cancer cells, which may increase survival and resistance to restriction of cell growth. This hypothesis is supported by clinical observations that cell lines exhibiting elevated Hsp27 are resistant to certain chemotherapeutic drugs such as doxorubicin, colchicine, and vincristine (34), and by a study demonstrating that transfection of breast cancer cells with exogenous Hsp27 resulted in elevated resistance to doxorubin, whereas overexpressing antisense *HSP27* rendered cells more sensitive to the drug (62). Early detection and treatment of potentially metastatic cancer is crucial for survival, and understanding in detail the contributions Hsps make to the cancer state may be of consequence when deciding therapeutic regimens.

Programmed cell death, or apoptosis, is a critical component of organismal development, but can also be engendered by severe cellular stresses, such as heat shock. Heat shock-induced apoptosis is also subject to regulation: the proto-oncogene c-*myc* potentiates cell killing (47) whereas expression of the anti-apoptotic gene bcl-2 reduces thermosensitivity by blocking the apoptotic response (7,78). The recent generation of an *hsf1* null mutation in cultured embryonic cells has led to the discovery that the heat shock response is essential for resisting heat-induced apoptosis. $HSF1^{(-/-)}$ cells exhibit a profound loss of thermotolerance and rapidly undergo apoptosis after lethal heat shock, as demonstrated by flow cytometric analysis and TUNEL assay (53). These data, together with the finding that the developmentally regulated *hsp70-2* is required for the prevention of apoptosis in spermatocytes (21), suggest cross-talk between cellular survival and death mechanisms. The inability to mount a protective response during stress, or activate molecular chaperone activity during crucial stages in development, may be interpreted by the cell as irreparable defects that in turn elicit the apoptotic response.

CONCLUSIONS AND PERSPECTIVES

In the years since its discovery, the heat shock response system has metamorphosed from a cytological observation of "puffs" on *Drosophila* salivary gland chromosomes to a paradigm for eukaryotic gene regulation by transcriptional control. This paradigm is further expanding to include a variety of unexpected associates involved in inducing and regulating HSF function. The recruitment of molecular chaperone function through HSF activation for multiple aspects of cell biology, including division, development, and

differentiation, is an expanding area of research to which can be brought to bear all the tools and knowledge gained from the sizable body of preceding work. Recent technological advances in analysis of gene expression, such as DNA microarrays and SAGE (Serial Analysis of Gene Expression) analysis, coupled with the increasing availability of genomic data, will allow for whole-genome interrogation of transcriptional responses to environmental signals. These approaches have the added benefit of uncovering, in a single experiment, large numbers of unknown, coordinately controlled target genes.

A central theme that resonates throughout the varied aspects of HSF and Hsp function and heat shock gene transcription is the remarkable conservation that exists across the entire eukaryotic lineage, from humans to single-celled yeasts. Mutational analysis of the heat shock response in yeast has long contributed to the field, while the phenotypic consequences of loss of HSF function are only now being explored in higher eukaryotic cells. Moreover, abstraction of the complex vertebrate HSF gene family into the simplified yeast milieu has allowed new insight into the diversity of these isoforms, such as the demonstration that HSF2 can, in fact, respond to heat stimuli, even though it has not been shown to in its native cell (48). Lastly, it is likely that HSF/Hsp requirements for basic cellular processes like signal transduction through kinase cascades and cell cycle regulation will be recapitulated in higher eukaryotic cells. Because defects in many of these processes translate into disease states such as cancer in humans, this information will be of vital importance.

ACKNOWLEDGMENTS

K. Morano was supported by the Cancer Biology Training Program at the University of Michigan Comprehensive Cancer Center (NIH 5T32CA09676-06) and a National Institutes of Health NRSA (1F32 GM19195-01). We also gratefully acknowledge support from the Taisho Excellence in Research Program, Taisho Pharmaceuticals Co., Ltd, Japan, and the University of Michigan Comprehensive Cancer Center. We thank the American Society for Microbiology for permission to reprint a portion of Fig. 4. D. J. Thiele is a Burroughs Wellcome Toxicology Scholar.

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