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7. J.C.L. Plumier et al., *J. Clin. Invest.* **95**, 1854–1860 (1995).
8. R.J. Heads, D.M. Yellon, and D.S. Latchman, *J. Mol. Cell. Cardiol.* **27**, 1669–1678 (1995).
9. M.J.D. Wagstaff et al., *J. Biol. Chem.* **274**, 5061–5069 (1999).
10. R. Morimoto, *Science* **259**, 1409–1410 (1993).
11. A. Stephanou and D.S. Latchman, *Gene Expr.* **7**, 311–319 (1999).
12. R.N. Haire, M.S. Peterson, and J.J. O'Leary, *J. Cell Biol.* **106**, 883–891 (1988).
13. D.M. Yellon and D.S. Latchman, *J. Mol. Cell. Cardiol.* **24**, 113–124 (1992).
14. R.A. Young, *Annu. Rev. Immunol.* **8**, 401–420 (1990).
15. V.B. Dhillon, D.S. Latchman, and D.A. Isenberg, *Lupus* **1**, 3–8 (1991).
16. S.E. Conroy and D.S. Latchman, *Br. J. Cancer* **74**, 717–721 (1996).

ADDITIONAL READING

Latchman D.S., ed., *Stress Proteins*, Springer-Verlag, New York, 1998.

HEAT SHOCK RESPONSE: CELLULAR AND MOLECULAR RESPONSES TO STRESS, MISFOLDED PROTEINS, AND DISEASES ASSOCIATED WITH PROTEIN AGGREGATION

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The molecular response to environmental and physiological stress serves to alert cells to imminent danger and to protect the genetic and biosynthetic apparatus from sustaining potentially lethal damage. The "heat shock response" functions as a stress-sensor of proteotoxicity by modulating the levels of molecular chaperones proportional to the stress, thus preventing the accumulation of misfolded proteins and protein aggregates that interfere with normal cellular function. Exposure to acute and toxic environmental stressors including extreme temperatures, ultraviolet (UV) light, oxidants, toxic chemicals, pharmacologically active molecules, mutagens, and the expression of mutant proteins results in the induced expression of heat shock proteins and molecular chaperones that function to reestablish protein homeostasis. Consequently, many of these stresses, if prolonged, result in the chronic expression of heat shock proteins, which leads to cellular dysgenesis and pathologies associated with tissue injury and repair in diseases including stroke, ischemia and myocardial reperfusion damage, cancer, and neurodegenerative diseases.

The heat shock response represents a genetically regulated process, initiated by exposure to diverse forms of environmental and physiological stress, that results in the activation of genes encoding molecular chaperones, proteases, and other inducible genes that function during protection and recovery from stress (1–4). Although the exposure of cells and organisms to heat shock may have origins with the stress associated with evolution in the primordial environment, the heat shock response has since become associated with a diverse array of physiological and biochemical stresses, including the cellular response to infection with viral and bacterial agents, exposure to transition heavy metals, amino acid analogs, pharmacologically active small molecules, and oxidants. Common to these stresses are effects on protein biogenesis defined by events associated with protein synthesis, folding, translocation, assembly, and degradation. Stress challenges protein homeostasis and results in an increased flux of nonnative proteins, which if left unprotected have an increased propensity to misfold and self-associate to form protein aggregates. Consequently, the heat shock response through the elevated synthesis of molecular chaperones and proteases responds rapidly and precisely to the intensity and duration of specific environmental and physiological stress signals by repairing protein damage to reestablish protein homeostasis.

Studies on the heat shock response have revealed how the cell senses stress and established the role of heat shock proteins in preventing and repairing protein damage. While prolonged exposures to extreme stress are harmful and can result in cell and tissue death, activation of the heat shock response by prior exposure to heat shock (and other stresses) results in stress tolerance and cytoprotection against subsequent bouts of stress-induced molecular damage (5). Transient exposure to intermediate elevated temperatures or reduced levels of chemical stress cross-protects cells, tissues, and organisms against sustained, normally lethal, exposures to stress. This reveals a valuable survival strategy that "a little stress is good." Moreover, stressed cells are often cross-protected from exposure to other forms of stress; this reveals an important aspect of the role of stress responses in adaptation and survival.

The complexity that underlies the diverse array of stress conditions resulting in the elevated expression of heat shock genes can be classified into four major categories (Fig. 1) (1) environmental stresses—heat shock, amino acid analogs, drugs, oxidative stress, toxic chemicals, heavy metals, and pharmacologically active small molecules; (2) nonstress conditions—the cell cycle, growth factors, serum stimulation, development, differentiation, and activation by certain oncogenes; (3) physiological stress and disease states—neuroendocrine hormones, tissue injury and repair, fever, inflammation, infection, ischemia and reperfusion, and cancer; and (4) diseases of protein aggregation—Huntington's disease, Alzheimer's disease, Parkinson's disease, and ALS. For each of these categories, the various conditions indicated are typically associated with the overexpression of one or more heat shock proteins through activation of heat shock factor (HSF) and the heat shock response.

This chapter will address (1) the role of molecular chaperones in protein biogenesis, with an emphasis on protein folding, translocation, and protection against the deleterious consequences of misfolded proteins; (2) the role of the heat shock response and heat shock proteins in cytoprotection against disease and as stress sensors; and (3) the role of the heat shock response as the molecular response to stress to ensure the rapid expression of genes that encode heat shock proteins and molecular chaperones.

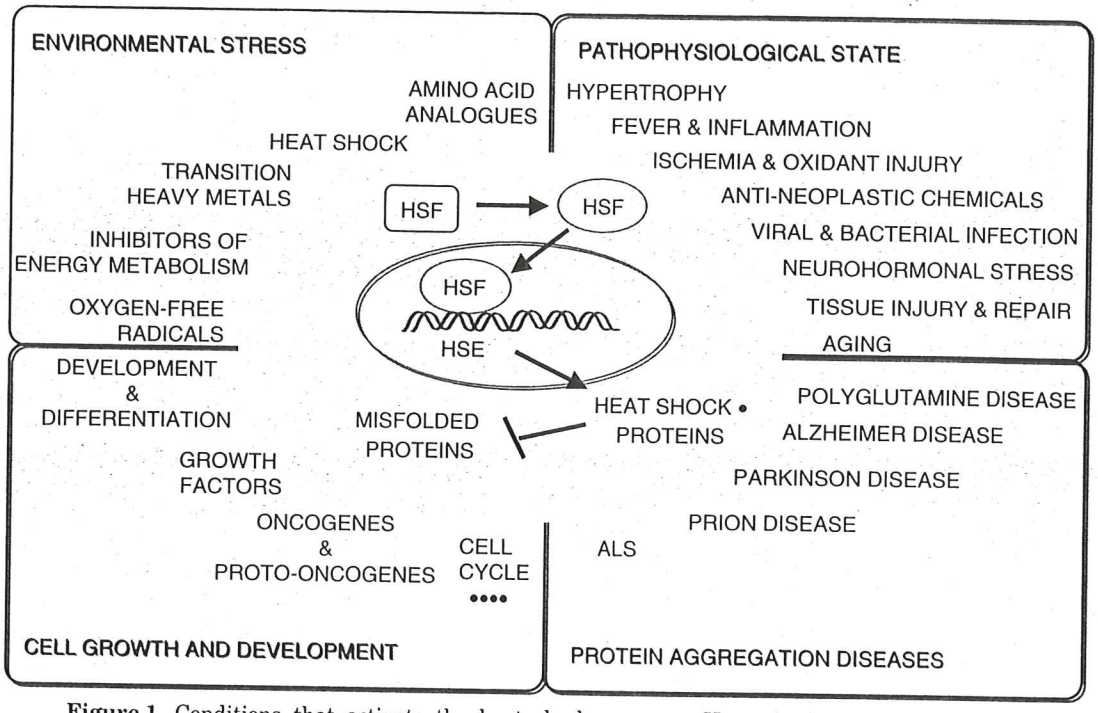

Figure 1. Conditions that activate the heat shock response. Heat shock gene expression is induced environmental and physiological stress, nonstressful conditions including cell growth and development pathophysiological states, and protein aggregation diseases, conditions that are associated with the appearance of misfolded proteins. Activation of the heat shock transcription factor leads to the expression of heat shock proteins, which function to prevent and repair protein damage through interaction with the misfolded proteins.

MOLECULAR CHAPERONES: ROLES IN PROTEIN BIOGENESIS AND THE RESPONSE TO MISFOLDED AND AGGREGATION-PRONE PROTEINS

General Features and Biochemical Properties of Molecular Chaperones

Although some proteins have the ability to refold spontaneously, in vitro, when diluted at low concentrations from denaturants, larger, multidomain proteins often have a propensity to misfold and aggregate. The challenge, in vivo, within the density packed environment of the cell is to ensure that nascent polypeptides fold properly, translocate, and assemble as multimeric complexes, and moreover that nonnative intermediates that accumulate during normal biosynthesis or are enhanced due to mutations or environmental stress are efficiently captured, refolded, or degraded. Molecular chaperones of the Hsp104, Hsp90, Hsp70, and Hsp60 class accomplish this by capturing nonnative intermediates and, together with cochaperones and ATP, facilitate the appearance of the folded native state (3). As will be described in detail, the Hsp70 chaperones recognize hydrophobic residue-rich stretches in polypeptides that are transiently exposed in folded intermediates and typically confined to the hydrophobic core in the native state (6). This contrasts with the Hsp60/GroEL chaperonin, which creates a protected environment with the properties of a "protein-folding test tube" in which nonnative proteins undergo rounds of binding and release to acquire the native state (7,8). Common to these chaperone interactions is their ability to shift the equilibrium of protein folding toward on-pathway events and to minimize the appearance of nonproductive intermediates that may have a propensity to aggregate as misfolded species.

The family of molecular chaperones is large, diverse in size and apparent structure, and yet highly conserved throughout evolution (1,3,9). Most chaperones are abundant in growing cells and can attain concentrations of 5 to 20% of total cell protein. They are classified according to molecular size, that is, Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and small Hsps distributed in all subcellular compartments (Table 1). Biochemical studies on chaperones have established common properties of Hsp104, Hsp90, Hsp70, the small Hsps, immunophilins (FKPB52 and CyP40), the steroid aporeceptor protein p23, and Hip (Hsp70 and Hsp90 interacting protein) to prevent the in vitro aggregation of model protein substrates and to maintain the substrate in an intermediate folded state competent for subsequent refolding to the native state (10–13). A distinction among proteins that exhibit the properties of chaperones is that refolding to the native state requires the activity of a specific subset of chaperones such as Hsp90, Hsp70, or Hsp60/GroEL and nucleotide. Cycles of nucleotide binding and hydrolysis are regulated by a large family of cochaperones such as p23 or the immunophilins, which enhance Hsp90; dnaJ, Hip, or Bag proteins, which associate with Hsp70; and Hsp10/groES, which stimulates Hsp60/groEL (Fig. 2) (10,14,15). The association of chaperones with different partner proteins thus influences the folded state of the substrate and consequently its activities.

The Hsp70 Family of Molecular Chaperones

The Hsp70 family of chaperones is highly conserved among nearly all prokaryotes and all eukaryotes. Hsp70s consist of

Table 1. Brief Summary of the Nomenclature, Location, and Function of the Major Heat-Shock Protein (HSP) Families

Family	Organism	Chaperones	Location	Functions[1]
HSP100	*E. coli*	ClpA,B,C	cytosol	Role in stress tolerance; helps the resolubilization of heat-inactivated proteins from insoluble aggregates.
	S. cerevisiae	HSP104	cytosol	
HSP90	*E. coli*	HtpG	cytosol	Role in signal transduction (e.g., interaction with steroid hormone receptors, tyrosine kinases, serine/threonine kinases); refolds and maintains proteins in vitro; autoregulation of the heat shock response; role in cell cycle and proliferation.
	S. cerevisiae	HSP83	cytosol	
	Mammals	HSP90	cytosol	
		GRP94	endoplasmic reticulum (ER)	
HSP70	*E. coli*	DnaK	cytosol	Roles in lambda phage replication; autoregulation of the heat shock response; interaction with nascent chain polypeptides; functions in interorganellar transport; roles in signal transduction; refolds and maintains denatured proteins; role in cell cycle and proliferation; antiapoptotic activity; potential antigen-presenting molecule in tumor cells.
	S. cerevisiae	Ssa 1-4	cytosol	
		Ssb 1,2	cytosol	
		Kar2	ER	
		Ssc1	mitochondria	
	Mammals	HSC70	cytosol/nucleus	
		HSP70	cytosol/nucleus	
		BIP	ER	
		mHSP70	mitochondria	
HSP60	*E. coli*	groEL	cytosol	Refolds and prevents aggregation of denatured proteins in vitro; may facilitate protein degradation by acting as a cofactor in proteolytic systems; role in the assembly of bacteriophages and Rubisco (an abundant protein in the chloroplast)
	S. cerevisiae	HSP60	mitochondria	
	Plants	Cpn60	chloroplasts	
	Mammals	HSP60	mitochondria	
HSP40	*E. coli*	dnaJ	cytosol	Essential cochaperone activity with HSP70 proteins to enhance rate of ATPase activity and substrate release.
	S. cerevisiae	Ydj1	cytosol/nucleus	
	Mammals	Hdj-1		
Small HSPs	*E. coli*	lbp A and B	cytosol	Suppresses aggregation and heat inactivation of proteins in vitro; confers thermotolerance through stabilization of microfilaments; antiapoptotic activity.
	S. cerevisiae	HSP27	cytosol	
	Mammals	αA and αB-crystalline	cytosol	
		HSP27		

[1]Ref. 1, 2, 3, 5, and 9

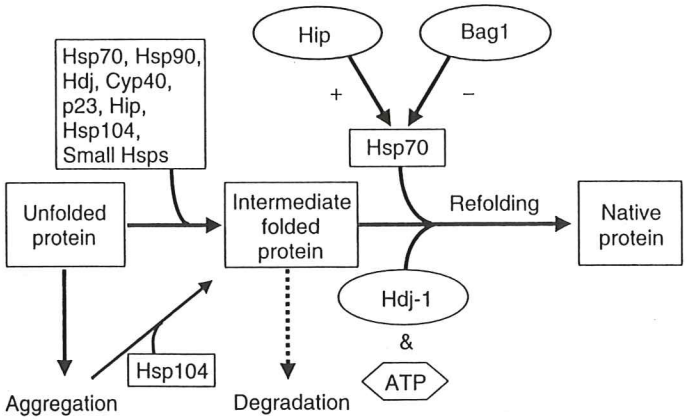

Figure 2. Chaperone networks and the regulation of protein conformation. The biochemical fate of an unfolded protein is schematically presented. In absence of chaperones, unfolded proteins are prone to aggregation. Presence of the molecular chaperones Hsp70, Hsp90, Hdj. Cyp40, p23, Hip, Hsp104, or small Hsps results in intermediate folded state. The chaperones prevent aggregation or target the unfolded proteins for degradation. Proteins in an intermediate folded state can be refolded to the native state by Hsp70, ATP, and the chaperone Hdj-1. Refolding can be positively (+) or negatively (−) influenced by Hsp70 cochaperones like Hip and Bag1.

a conserved amino-terminal 42-kD ATPase domain, a less well conserved 18-kD peptide-binding domain, and a carboxyl terminal 10-kD variable domain whose function is poorly characterized. Central to the different properties of Hsp70s is the regulation of the substrate binding affinity and release cycle by ATP-induced conformational changes. The substrate recognition properties of Hsp70 were characterized by studies on the prokaryotic Hsp70, DnaK, and the lumen localized Grp78 or BiP (6,16). Association with DnaK requires that proteins be in an extended conformation with the recognition site containing a core of five hydrophobic residues flanked by two positively charged amino acid residues. Likewise, recognition by mammalian Hsc70 requires a minimum of seven hydrophobic or hydrophobic-basic residues (17). The hydrophobic residues required for Hsp70 interaction correspond to regions that are typically found within the interior of folded proteins. This property of Hsp70, the binding and release of nonnative polypeptides, emphasizes the central role of this chaperone to prevent protein misfolding and aggregation (6).

The substrate binding and release cycle of Hsp70 requires the activities of cochaperones. For example, in the reaction cycle of DnaK, the substrate is delivered by the Hsp40 family member DnaJ, resulting in the substrate: DnaK complex. Subsequent exchange of ADP for ATP by GrpE is required

to release the substrate from DnaK (18,19). Mammalian cytosolic Hsp70 and Hsc70 require the nucleotide-stimulating properties of Hsp40/Hdj-1 for protein folding; however, the reaction cycle does not appear to depend on a nucleotide-exchange factor like GrpE (20,21).

In addition to Hsp40, the mammalian cytosolic Hsp70s can also be influenced by additional coregulators. These coregulators have been identified by interaction cloning approaches and have been characterized in vitro for their influence on Hsp70 chaperone activity. Hip, initially cloned as a glucocorticoid receptor binding protein, binds to the ATPase domain of Hsp70, stabilizes ADP-bound conformation with a high affinity for the substrate, and stimulates Hsp70-dependent refolding of unfolded model substrates. Bag1 and Chip, in contrast, inhibit Hsp70 chaperone activity (22–26). Bag1, which competes with Hip, binds to the ATPase domain of Hsp70 and accelerates nucleotide exchange. Consequences of this acceleration on the Hsp70-substrate complex are still unresolved, and either results in the formation of a stable Bag1/Hsp70/substrate complex or a premature release of the substrate. Chip inhibits by binding to the C-terminal domain and blocking ATP hydrolysis, which keeps Hsp70 in its low substrate affinity form (22).

It is likely that the outcome of Hsp70/cochaperone/substrate interactions differ in vivo from in vitro. The presence of other cellular factors, for example, the presence of the degradation machinery or additional Hsp70 regulatory proteins, could shift the fate of Hsp70 substrates. This suggestion has been supported by several studies. For example, the Hsp70 cochaperone Hop, which binds to the carboxyl terminal domain of Hsp70, can negate the inhibitory influence of BAG-1 on Hsp70-dependent refolding by direct communication with amino-terminus of BAG-1. In addition, BAG-1 has been found to stimulate association of Hsp70 with the 26S proteasome, which could make degradation an alternative fate for BAG-1/Hsp70-bound substrates in a cellular context (27).

Under normal growth conditions, Hsp70s are involved in biosynthetic events in which proteins are partially or completely unfolded. Examples of these processes include protein translation, protein translocation across intracellular membranes, and protein degradation. While proteins are synthesized at the ribosome, Hsp70s are associated with the nascent chains, which is thought to prevent the aggregation of these chains and keep polypeptides in a folding competent conformation until complete domains are translated and able to fold (28,29). Similarly, Hsp70s assist in translocation of precursor proteins across the intracellular membranes of the endoplasmic reticulum and the mitochondria (30). Translocation requires the activities of both cytosolic and organellar Hsp70s. Hsp70s also have a role in protein degradation; depletion of the level of the mammalian cytosolic chaperones Hsp70 and Hsc70 reduces proteasomal degradation of proteins such as actin and α-crystallin (31). How Hsp70s assist in protein degradation has not been resolved, but it has been proposed that Hsp70s have a role in presenting substrate proteins to the proteolytic apparatus in a partially unfolded conformation permissive for degradation (32).

Under stress conditions, Hsp70 is required for survival of mammalian cells. Reduction in the levels of Hsp70 by injection of antibodies against Hsp70 or by competitive inhibition at the level of transcription, decreases cell survival after heat shock (33,34). Depending on the temperature and duration of the exposure, heat stress can cause damage to nearly all cellular structures and functions, including the transcriptional machinery, ribosome assembly, and the cytoskeleton. Constitutive overexpression of Hsp70 increases cellular heat stress resistance and causes an accelerated recovery of RNA transcription, ribosome assembly, and microtubule formation during recovery from stress (35–37). It is likely that Hsp70 protects and repairs these cellular functions through its activities as a chaperone and by interactions with stress-unfolded proteins.

ROLE OF HEAT SHOCK PROTEINS IN STRESS-INDUCED CYTOPROTECTION AND DISEASE

The abnormal expression of heat shock proteins has been extensively documented in a growing number of diseases, including ischemia and reperfusion damage, cardiac hypertrophy, fever, inflammation, metabolic diseases, bacterial and viral infection, cell and tissue injury, aging, cancer, and protein misfolding diseases (1,38). Serum antibodies to Hsp90, Hsp70, Hsp60, and small heat shock proteins have been detected in individuals with infectious diseases and autoimmune diseases, including rheumatoid arthritis and insulin-dependent diabetes (39). These observations have led to questions whether stimulation of the immune response results from or is the cause of cell damage. Nevertheless, the appearance of circulating antibodies to heat shock proteins is a measure of the organismal response to stress and damage. Although the acute response to stress may be critical for recovery and long-term survival, the chronic expression of heat shock proteins as occurs in tissues exhibiting a pathological state may be deleterious for protein biogenesis and have long-term negative effects on cell growth.

Cytoprotection and repair against the deleterious effects of stress and trauma can be accomplished by the overexpression of one or more heat shock protein genes. Yeast cells engineered to overexpress Hsp70, the small heat shock proteins, or Hsp 104 are cross-protected against exposures to otherwise lethal heat shock temperatures, and high doses of H_2O_2, heavy metals, arsenite, anoxia, and ethanol (5). Likewise, in vertebrates, modulation of the heat shock response and overexpression of specific heat shock proteins has been shown to limit or prevent tissue damage associated with certain chronic diseases.

Metabolic Stress During Ischemia and Reperfusion

Metabolic injury as occurs during ischemia of the brain, kidney, heart, and liver results from decreased blood flow to these tissues (40). The pathology associated with ischemia is due to both the molecular injury during chronic adaptation and the subsequent attempt to adapt to intervention, which can result in oxidative stress as nutrients are rapidly replenished to the diseased tissue. Expression of heat shock proteins is rapidly induced, both during ischemia and to an even greater degree on reperfusion associated with the appearance of oxygen-free radicals (41). Exposure to environmental toxins such as xenobiotics and aromatic hydrocarbons that promote oxidative damage could potentiate the damaging effects of ischemia and reperfusion. In the pathology of myocardial disease, the induction of Hsp70 has been associated with both ischemia and reperfusion and therefore could reflect

the appearance of damaged proteins during adaptation of the myocardium (42–44). The elevated synthesis of heat shock proteins, therefore, could reflect the response of myocardiocytes to survive stress by repairing protein damage. A correlation between the levels of Hsp70 and the degree of myocardial protection has been demonstrated. Moreover, transgenic mice overexpressing Hsp70 exhibited an enhanced resistance to myocardial ischemic stress, thus providing direct evidence for a role of heat shock proteins in cytoprotection (42–44). Therefore a potential strategy would be to enhance the expression of heat shock proteins such as Hsp70, which in turn may confer a more rapid reestablishment of normal cardiac protein synthesis and myocardial function. A similar strategy could also be considered for other diseases associated with chronic nutrient or oxygen deprivation.

Stress and Oncogenesis

Many human tumors express increased levels of one or more heat shock proteins, which in many cases is associated with poor prognosis and resistance to therapy. The increased expression of heat shock proteins in tumors raises questions whether they have an active role in events leading to deregulation of cell growth and tumor formation. Alternatively, the expression of heat shock genes could reflect cellular adaptation to expression of mutant proteins, changes in the cellular protein composition, and significant changes in the microenvironment of a tumor. In breast tumors, the level of Hsp70 expression correlates with short-term disease-free survival, metastasis, and poor prognosis among patients treated with combined chemotherapy, radiation and hyperthermia (45,46). Increased levels of Hsp90α, Hsp90β, Hsp60, and Hsp27 have been described for breast tumors, lung cancer, leukemias, and Hodgkin's disease (47–49). Several observations suggest that heat shock proteins may have an active role in enabling and maintaining the tumorigenic properties of cancer cells. Gene transfer–mediated overexpression of Hsp70 and Hsp27 by stable transfection in cultured cells or in transgenic animals results in transformation and tumor formation (50,51). Overexpression of Hsp70 alone in primary cells can lead to transformation (52); when Hsp70 expression is turned off, the transformed phenotype is reversed. Likewise, transgenic mice expressing human Hsp70 develop T cell lymphomas (52). One observation suggests that maintaining high concentrations of Hsps may even be a prerequisite for tumor cell survival. Adenoviral transfer or transfection of antisense Hsp70 induces cell death in human breast cancer cells, whereas normal breast epithelial cells or fibroblast are not affected (53).

What is the molecular mechanism through which heat shock proteins could potentiate the transformed phenotype? One possibility is that overexpression of certain chaperones alters the conformation or activities of key regulatory proteins or mutated tumor suppressor proteins (54). These alterations then potentiate the transforming activity of oncogenes, or interfere with stress signaling, thus preventing a cellular defense mechanism that would lead normally to the elimination of the transformed cells by apoptosis. Examples of such proteins are components of the cell cycle machinery, kinases, hormone receptors, and other proteins implicated in cancer progression. Hsp90 interacts with tyrosine kinase oncogene products pp60-v-src, fes, and fgr to form highly stable complexes (55–58). This interaction alters the half-life of pp60-v-src and modulates both its kinase activity and its specificity (54), leading to the proposal that altered levels of Hsp90 detected in tumor cells may be associated with the oncogenic activity of the kinase. Hsp70 has been detected in complexes with proteins, including SV40 large T antigen, adenovirus E1A protein, cellular *c-myc*, and the tumor suppressor protein p53 (59–61). Likewise, overexpression of Hsp70 can suppress transforming property of p53 (54). This could suggest that sequestration of mutant p53 by the chaperone reduces the opportunity for wild-type and mutant p53 to associate, thereby allowing the wild-type protein to perform its antiproliferative function. The association of mutant p53 and Hsp70 could lead to conformational diversity affecting the nature of other mutant p53 substrate interactions (62). Further understanding of these mechanisms may be of great interest for the development of anticancer strategies targeted to Hsp70 overexpression as a means to suppress p53-induced transformation.

Changes in the level of heat shock proteins, reflecting adaptation to a stressed state, shift the balance between proliferative signals and the events leading to cell death. The Hsp70 chaperone network (Hsp70 and BAG1) functions in a stress signaling pathway to negatively regulate cell growth

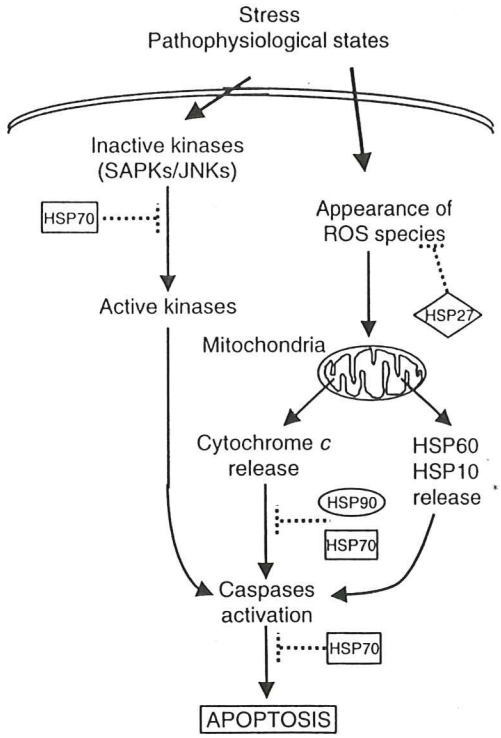

Figure 3. Heat shock proteins act at multiple points in the apoptotic pathway Stresses, such as UV irradiation and heat shock, and pathophysiological conditions can induce apoptotic death by at least two pathways, which can be blocked by Hsps at multiple stages. One pathway is through appearance of reactive oxygen species (ROS), which cause the release of cytochrome *c* from mitochondria subsequent caspase activation. Hsp27 can act to neutralize the effects of reactive oxygen species (ROS), preventing the release of cytochrome *c* from the mitochondria. The activation of caspases in response to cytochrome *c* release can be prevented by Hsp70 or Hsp90. Hsp70 can inhibit in addition by blocking metal events downstream of caspases activation. A second pathway to induce apoptosis is through the activation of the stress kinases SAPKs/JNKs. Hsp70 can also prevent apoptosis by blocking the activation of SAPKs/JNK.

during the stress response (63). The Hsp70 cochaperone BAG1 also functions as a positive regulator of the MAP kinase pathway by direct interaction with Raf1 kinase, and in a Ras-independent mechanism, activates the events leading to cell growth. By direct protein–protein interaction, BAG1 becomes sequestered in a heteromeric complex with Hsp70, such that during stress when Hsp70 levels increase, Raf1 kinase is inactivated, thus linking cell growth with cell stress. As stress conditions inevitably must signal to the cell death machinery, there is increasing evidence that heat shock proteins function at multiple points in apoptosis (Fig. 3). Elevated levels of heat shock proteins (Hsp90, Hsp70, and Hsp27), attained by transient transfections or under the control of tetracycline-inducible promoters, inhibit apoptosis by reducing or blocking caspase activation and by suppressing mitochondrial damage and nuclear fragmentation (64,65). In this scenario, both Hsp90 and Hsp70 have been shown to prevent the assembly of a functional apoptosome (66,67). In addition, Hsp70 appears to act at an earlier step on the apoptotic pathway by preventing JNK activation (64,68). In the case of tumor necrosis factor (TNF)-induced apoptosis, Hsp70 rescues cells from apoptosis downstream of JNK activation, even downstream of the activation of effector caspases (69). Complementary observations have been made for Hsp27, such that overexpression of Hsp27 has been shown to block apoptosis induced by heat, H_2O_2, Fas ligand, and anticancer drugs (70,71). However, Hsp27 does not confer resistance to other stresses such as lymphokine-activated killer cells or UV radiation; these results reveal that different chaperones are not redundant in protection against stress-induced cell death.

Another recent development associated with heat shock proteins and cancer has been the development of heat shock protein–based vaccines against tumor antigens. The rationale for this follows from observations that a subset of the heat shock proteins are detected antigenically on the surface of certain tumor cells, where they can activate the immune response resulting in elimination by the immune system (72,73). Indeed, heat shock protein (HSP)-peptide complexes isolated from murine cancers elicit protective immunity and T lymphocytes specific for the cancer from which the HSPs are isolated (74–76), although suppression of subsequent metastases was variable (75,76). The search for specific tumor antigens continues to be a major objective for cancer therapy, so the use of HSP-peptide complexes offers a novel source of tumor-specific antigens that could be used to target tumor cells for drug delivery or for elimination by the host immune system.

Protein Misfolding and Neurodegenerative Diseases

A growing number of human neurodegenerative disorders appear to be associated with the accumulation of misfolded proteins and the appearance of protein aggregates, fibrils, or plaques. These include inherited disorders caused by CAG/polyglutamine expansion, as occurs in Huntington's disease (HD), Kennedy disease, the spinocerebellar ataxias SCA1, SCA2, MJD (SCA3), SCA6, and SCA7, and dentatorubral-pallidolluysian atrophy (DRPLA), Alzheimer's disease and prion disease (77). A hallmark of these diseases is the expression of abnormal proteins that form a predominant β-sheet conformation, which self-associates to form insoluble aggregates in neuronal cells or in the extracellular space (78–80).

Prion diseases from yeast to humans are associated with changes in the folded state of the prion, which correlates with conversion from a noninfective to an infective state. Among the fascinating features of prion diseases is the epigenetic transmission of an altered protein conformation, thus endowing the infectious prion with unique biological properties. The association of chaperones with prion disease has been suggested based on studies in the yeast *Saccharomyces cerevisiae*. A subunit of a translation termination factor, Sup35p, can form self-seeded fibers in [psi+] strains, in a prion-like manner, which is prevented if the heat shock protein Hsp104 is deleted or overproduced (81). Hsp104 can directly interact with the prion-like domain of Sup35p and with the human prion protein, which is suggestive for a conserved mechanism (82). The conserved properties of heat shock proteins and the striking similarity in the characteristics of in vitro formed fibers of Sup35p, polyglutamine repeat, and prion proteins furthermore has strongly suggested the involvement of heat stock proteins in aggregate formation in other neurodegenerative disorders.

Indeed, the heat shock response and heat shock proteins have been implicated in these polyglutamine expansion misfolding diseases. Studies with mammalian tissue culture cells and the nematode *Caenorrhabditis elegans* have established that the heat shock response is activated in cells expressing polyglutamine expansion–containing proteins (83,84). Moreover, the colocalization of several heat shock proteins, including the Hsp40 family members Hdj-1 and Hdj-2, Hsp70 and ubiquitin, with polyglutamine aggregates in mouse tissues and tissue culture cells has suggested a direct relationship between these heat shock proteins and polyglutamine diseases (85). Recently, evidence has accumulated that heat shock proteins could play important roles in cytoprotection based on observations that overexpression of Hdj-1, Hdj-2, or Hsp70 reduces polyglutamine aggregate formation and prevents cellular degeneration (86–88). These observations offer interesting possibilities to develop therapeutic strategies based on activation of the stress response or selectively increasing the levels of individual chaperones.

STRESS-INDUCED TRANSCRIPTIONAL REGULATION OF HEAT SHOCK GENES

Family of Heat Shock Factors

In higher eukaryotes, the stress-induced regulation of the heat shock response occurs principally by activation of a family of heat shock transcription factors (HSFs) from an inert state to transcriptionally competent DNA binding state (89–92). Among eukaryotes, the complexity of HSF-dependent regulation varies among species with yeast, *C. elegans*, and *Drosophila* encoding a single HSF gene (L.-J. Tai, J. Morley, and R. Morimoto, personal communication; 93–95) to vertebrates in which four HSF genes (HSF1 to 4) have been isolated and characterized (96–100).

Comparison of the various cloned HSF genes reveals an overall sequence identity of 40% with a high degree of structural conservation in the DNA binding domain (91,101,102), an adjacent 80–amino acid residue hydrophobic repeat (HR-A/B) necessary for the formation of homotrimers (95,103,104), the central region containing residues involved in negative regulation of HSF, and the carboxyl terminal transactivation domain (99,105–109). With the exception of HSF in budding yeasts and human HSF4, another

hydrophobic repeat (HR-C) is located adjacent to the carboxyl terminal transactivation domain and has been suggested to regulate trimer formation by interaction with HR-A/B (98,109–113). *Saccharomyces cerevisiae* HSF, unlike other HSFs, is not negatively regulated and is expressed constitutively as a DNA-binding competent trimeric species.

Of the HSFs expressed in vertebrate cells, HSF1 exhibits the properties most closely related to yeast and *Drosophila* HSF (114,115). In avian cells, HSF1 and HSF3 are coexpressed and coactivated by chemical and physiological stress suggesting functional redundancy, yet cells deleted for HSF3 are severely compromised for the heat shock transcriptional response; these results suggest that HSF1 and HSF3 exhibit a functional codependency (116–118). HSF3 has also been shown to interact with other transcription factors, such as the oncogene Myb, via direct protein-protein interaction with the HSF3 DNA binding domain (119). Myb is a growth-regulated transcription factor; consequently, the interaction between Myb and HSF3 suggests a new pathway for genetic crosstalk between cell growth and the stress response. Evidence is also accumulating to indicate that HSF1 may negatively influence the transcriptional activation of other cellular genes independent of direct binding to heat shock elements, perhaps via protein-protein interactions with HSF1 (120).

Another member of the HSF family, HSF2, regulates heat shock gene expression during cell growth, differentiation, and on deregulation of the protein degradative machinery. Activation of HSF2 was first observed in human erythroleukemia (K562) cells exposed to the differentiation agent hemin (121–123) and subsequently detected during murine spermatocyte differentiation (124) and embryogenesis (125,126). The stress-sensing pathway for HSF2 activation is associated with changes in protein degradation, in a cell-type independent manner, following exposure to inhibitors of the ubiquitin-dependent proteasome or in cells harboring conditional mutations in components of the proteasome machinery (127). The function of the ubiquitin-dependent proteasome machinery is to degrade short-lived and misfolded proteins; therefore, the relationship between HSF2 activity and the protein degradative machinery reveals a requirement for heat shock proteins, perhaps to prevent misfolding and aggregation of nonnative proteins targeted for degradation.

Regulation and Autoregulation of Heat Shock Factor

Stress-induced activation of HSF1 involves a multistep process (Fig. 4) involving translocation and relocalization within the nucleus, oligomerization to a trimeric DNA binding species, binding to the heat shock promoter elements, acquisition of a serine hyperphosphorylated state, and finally the induced transcription of heat shock genes (91,128–134). During prolonged exposures to heat shock, HSF1 activity attenuates as the transcription of heat shock genes return to control levels. Attenuation of the heat shock response is autoregulated and associated with fewer misfolded proteins in part due to the abundance and activities (134) of chaperones. During attenuation, HSF1 associates transiently with the molecular chaperones Hsp70 and Hdj1; these events correlate with the dissociation of HSF1 trimers from DNA, and refolding of the trimer to the monomer. Maintenance of HSF1 in a repressed state is delicately balanced and easily disrupted; for example, HSF1 that is transiently overexpressed is constitutively active rather than maintained in a repressed state; likewise, mutations in residues in the negative regulatory domain result in derepression of HSF1 activity (115,133). Acquisition of HSF1 DNA-binding activity is alone insufficient to activate transcription, as demonstrated by the effects of the anti-inflammatory drugs sodium salicylate, indomethacin, or ibuprofen, which induce DNA binding competent trimers that lack some of the posttranslational modifications associated with its transcriptional activity (135–137). However, rather

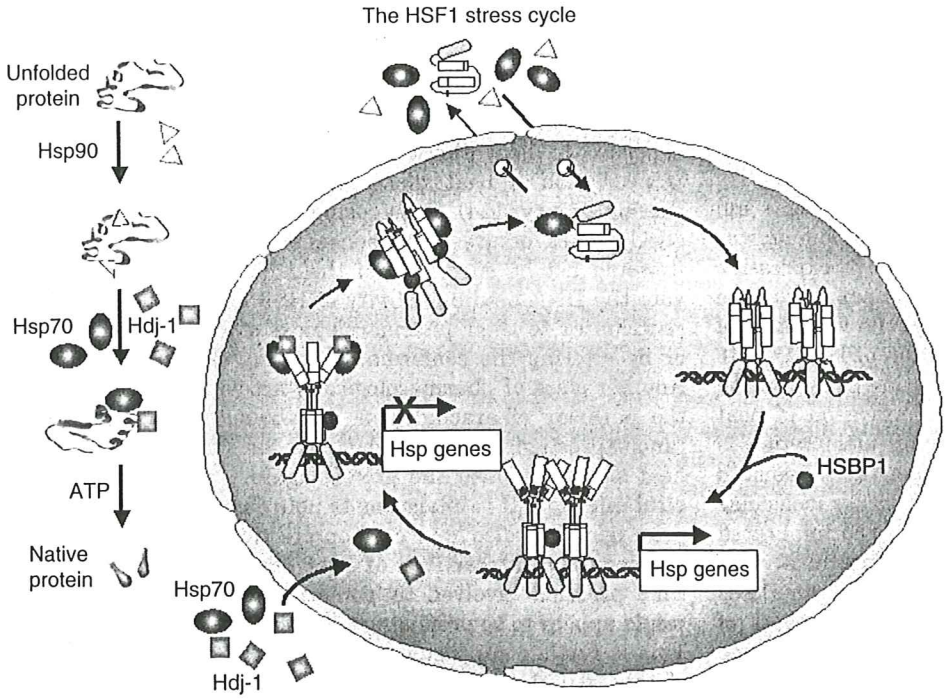

Figure 4. Regulation of the heat shock response. Activation of heat shock factor (HSF1) is linked to appearance of nonnative proteins and the requirement for molecular chaperones (Hsp90, Hsp70, and Hdj1 prevent the appearance of misfolded proteins. HSF1 exists in the control state as an inert monomer (shown intramolecularly negatively regulated for DNA binding and transcriptional activity) and undergoes step 1 activation to a DNA binding competent state that is transcriptionally inert, acquisition of induce phosphorylation resulting in complete activation and inducible transcription of heat shock genes, and attenuation of HSF1 activity. HSF1 activity is negatively regulated by heat shock factor binding protein 1 (HSBP1), which binds to the region of HSF1 corresponding to the heptad repeat, and by Hsp70 and Hdj1, which bind to transcriptional transactivation domain thus repressing HSF1 activity.

than corresponding to a nonfunctional form of HSF1, the salicylate-induced HSF1 can be converted in vivo to the fully active HSF1 by a subsequent exposure to intermediate heat shock temperatures (137).

The initial evidence for autoregulation of the eukaryotic heat shock response was based on the effect of amino acid analogs on gene expression in Drosophila cells. Azetidine, a proline analog, treatment results in the continuous activation of heat shock gene expression unlike the transient effects of heat shock (138). An explanation for this result was that amino acid analogs induce the heat shock response as a result of their incorporation into nascent polypeptides, which misfold. Such amino acid analog–containing nascent polypeptides would be expected to associate with Hsp70 and other chaperones (139); the sequestration of these permanently unfolded polypeptides by Hsp70 in turn results in activation of heat shock gene transcription. However, unlike the heat shock response where attenuation is linked to the de novo synthesis of heat shock proteins, the Hsp70 synthesized in amino acid analog–treated cells is likewise misfolded and therefore nonfunctional. Consequently, the heat shock response induced by amino acid analogs does not attenuate as the stress signal persists. These observations have led to the proposal that heat shock proteins are involved in autoregulation of the heat shock response. Genetic evidence to support the autoregulation of the heat shock response has shown that overexpression of the yeast Ssa1p (cytosolic Hsp70) dampens the heat shock response (140). Likewise, deletion of the yeast Hsp70 genes, ssa1 and ssa2, resulted in an unusually high level of expression of another Hsp70 gene (Ssa3p) and other heat shock proteins in a HSF-dependent manner (141). The relationship between HSF and Hsp70 was further supported by a search for extragenic suppressors of the temperature-sensitive phenotype of an Hsp70 mutant (ssa1 ssa2 strain), which also identified HSF as an interactive component of the regulatory response (142). A spontaneous mutant EXA3, which could reverse the growth defect of the Hsp70 mutant (ssa1 ssa2), is very closely linked to the gene encoding HSF and another mutation identified in the genetic screen maps to the HSF gene (143).

Biochemical evidence to support a role for molecular chaperones in the regulation of the heat shock response has demonstrated that stress-induced HSF1 trimers are associated with Hsp70, Hdj1 (Ddj1), and Hsp90 (144–149). The Hsp70-HSF complexes are sensitive to ATP and can be reconstituted in vitro (144,148). Through the use of HSF1 deletion mutants and direct in vitro binding assays, the transactivation domain was shown to interact with Hsp70 (148). Whereas overexpression of Hsp70 inhibited completely the induction of heat shock gene transcription, there was little or no effect on the formation of HSF1 trimers or on inducible phosphorylation of HSF1 (146). This demonstrates that Hsp70 can also function as a negative regulator of HSF1 activity and that the repression of heat shock gene transcription that occurs during attenuation is due to the repression of the HSF1 transactivation domain associated with Hsp70 binding (144,146,148). Other molecular chaperones such as Hdj1 or Hsp90 have also been implicated in the regulation of HSF1 activity. Hdj1 interacts with HSF1 in higher eukaryotes and also negatively regulates HSF1 transcriptional activity (148). A role for members of the DnaJ family in regulation of the heat shock response is also supported by observations in *S. cerevisiae* that the DnaJ homolog, SIS1, negatively regulates its own expression and that Drosophila Ddj1 associates with HSF1 (149,150). However, SIS1 autoregulation requires the heat shock element (HSE) and other sequences, suggesting that additional regulatory molecules might be involved. HSF may also associate with Hsp90; such interactions have been detected for yeast, rat, and rabbit Hsp90 (151) but not with human Hsp90 (145,146,148). Collectively, these results reveal that multiple chaperones are involved in the regulation of HSF1 and, moreover, that differences in specificity and kinetics may influence different aspects of HSF regulation and the heat shock response.

HEAT SHOCK RESPONSE AS A TARGET FOR NOVEL THERAPEUTICS

The heat shock response is the cell's natural defense against proteotoxicity. Consequently, the aberrant expression of heat shock proteins documented in a growing number of disease states, together with the compelling evidence that transient activation of the heat shock response establishes a cytoprotective state, suggests that efforts to control the expression or function of heat shock proteins could have therapeutic value. A pharmacological approach has been suggested from experiments using herbimycin-A as an inducer of Hsp70 with protective effects in simulated ischemia on rat neonatal cardiomyocytes (43) or the cytoprotective activity of a hydroxylamine derivative (Bimoclomol) during ischemia and wound healing (152).

Other classes of small molecules with heat shock regulatory properties include nonsteroidal anti-inflammatory drugs (NSAIDs), cyclopentanone prostaglandins, serine protease inhibitors, and inhibitors of the ATP-dependent ubiquitin-dependent proteasome (135,153). Salicylates and other NSAIDs have been shown to result in activation of HSF1 to an intermediate (in the activation pathway) that can be more readily activated by stress (135). Pretreatment with the NSAIDs sodium salicylate or indomethacin (at subthreshold concentrations) decrease the temperature threshold of the heat shock response; moreover, treatment with either drug alone confers cytoprotection (153). Exposure to aspirin or indomethacin at concentrations comparable to the clinical levels results in the priming of human cells for subsequent exposure to heat shock and other stresses, the enhanced transcription of heat shock genes, and cytoprotection from thermal injury (154). Considering that the expression of heat shock genes occurs in response to pathological conditions, it would not be surprising if the ability of NSAIDs to activate the DNA binding activity of HSF and Hsp70 expression contributes to their pharmacological efficacy, either directly or by altering the conformation of key signaling molecules. Another class of pharmacologically active small molecules in the pathway of arachidonate metabolism are the cyclopentanone prostaglandins, which have been shown to activate the heat shock response and protect against thermal injury and viral infection. It is intriguing to note that the cyclopentanone prostaglandins also have cardioprotective activity (155).

Given the interactions of heat shock proteins with multiple proteins involved in potentiating tumorigenesis, they would appear to be promising targets for anticancer therapies. This is further supported by the finding that neutralization of the activities of the stress-inducible Hsp70 by antisense

methods selectively kills breast tumor cells but not breast epithelial cells or fibroblasts. Similarly, the anticancer drug geldanamycin, a member of the benzoquinone ansamycin family of antibiotics (156), which acts by inhibiting cell proliferation and reverting oncogenic transformation (57,156), binds to the ATP-binding domain of Hsp90. By competitive inhibition of ATPase activity, geldanamycin has pleiotropic effects on Hsp90 substrate interactions and results in the decreased activity of essential key signal transduction proteins, including steroid receptors, cell cycle kinases, transcription factors, and p53 (157–162). Radicicol, an antifungal antibiotic with related structure, has the ability to suppress transformation by several oncogenes including Src and Ras, binds to the N-terminal domain of Hsp90, and also inhibits its chaperone activity (163). Based on these two promising drugs, Hsp90 has recently emerged as a promising target for anticancer therapy (162); however, it would not be unexpected that drugs targeting Hsp90 might have side effects on normal cellular function given the abundance and pleiotropic role of Hsp90.

The development of pharmacologically active small molecules that influence the regulation or function of heat shock proteins thus offers a novel approach to harness the positive cytoprotective properties of the heat shock response to adapt, repair, and survive the molecular damage associated with stress exposure.

CONCLUSION

- Exposure of cells to stresses such as heat shock, oxidant injury, toxic chemicals, and heavy metals results in an imbalance in protein metabolism that challenges the cell to respond rapidly, yet precisely, to minimize the deleterious effects of environmental and physiological stress.
- The heat shock response, through the activation of heat shock transcription factors, induces the expression of genes encoding molecular chaperones and proteases that function to sequester or degrade folded intermediates that appear in stressed cells.
- Diseases associated with environmental or physiological stress result in the deregulation of the heat shock response, resulting in pleiotropic effects on cell growth and cell death. The properties of chaperones as protein remodeling factors can alter the activities of many cellular proteins involved in cell signaling and gene expression.
- Accumulation of misfolded proteins, as occurs when mutant proteins are expressed or when cells are exposed to chronic stress, has been established to have a central role in "diseases of protein aggregation," including cystic fibrosis, prion diseases, Alzheimer's disease, Parkinson's disease, ALS, and Huntington's disease.

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BIBLIOGRAPHY

1. R.I. Morimoto et al., in R.I. Morimoto, A. Tissieres, and C. Georgopoulos, eds., *The Biology of Heat Shock Proteins and Molecular Chaperones*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1994, pp. 417–455.
2. B. Bukau, *Molecular Chaperones and Folding Catalyst, Regulation, Cellular Function and Mechanism*, Harwood Academic Publishers, Amsterdam, Netherlands, 1999.
3. F.U. Hartl, *Nature* **381**, 571–579 (1996).
4. C.P. Downes, C.R. Wolf, and D.P. Lane, Portland Press, London, U.K., pp. 168.
5. D.A. Parsell and S. Lindquist, in R.I. Morimoto, A. Tissieres, and C. Georgopoulos, eds., *The Biology of Heat Shock Proteins and Molecular Chaperones*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1994, pp. 457–494.
6. S. Rudiger, L. Germeroth, J. Schneider-Mergener, and B. Bukau, *EMBO J.* **16**, 1501–1507 (1997).
7. A. L. Horwich et al., *Cell* **74**, 909–917 (1993).
8. M. Mayhew et al., *Nature* **379**, 420–426 (1996).
9. M.J. Gething, *Molecular Chaperones and Protein-Folding Catalysts*, Oxford University Press, New York, 1997.
10. B.C. Freeman, D.O. Toft, and R.I. Morimoto, *Science* 274, 1718–1720 (1996).
11. U. Jakob, H. Lilie, I. Meyer, and J. Buchner, *J. Biol. Chem.* **270**, 7288–7294 (1995).
12. W.B. Pratt and M.J. Welsh, *Semin. Cell Biol.* **5**, 83–93 (1994).
13. D.F. Smith in *Molecular Chaperones and Protein-Folding Catalysts*, Oxford University Press, New York, 1997, pp. 518–521.
14. M.L. Freeman et al., *J. Cell. Physiol.* **164**, 356–366 (1995).
15. P. Goloubinoff, A.A. Gatenby, and G.H. Lorimer, *Nature* 337, 44–47 (1989).
16. G.C. Flynn, J. Pohl, M.T. Flocco, and J.E. Rothman, *Nature* **353**, 726–730 (1991).
17. A.M. Fourie, J.F. Sambrook, and M.J. Gething *J. Biol. Chem.* **269**, 30470–30478 (1994).
18. K. Liberek et al., *Proc. Natl. Acad. Sci. U.S.A.* **88**, 2874–2878 (1991).
19. A. Wawrzynow et al., *J. Biol. Chem.* **270**, 19307–19311 (1995).
20. B.C. Freeman, M.P. Myers, R. Schumacher, and R.I. Morimoto, *EMBO J.* **14**, 2281–2292 (1995).
21. Y. Minami, J. Hohfeld, K. Ohtsuka, and F.U. Hartl, *J. Biol. Chem.* **271**, 19617–19624 (1996).
22. C.A. Ballinger et al., *Mol. Cell. Biol.* **19**, 4535–4545 (1999).
23. J. Hohfeld, Y. Minami, and F.U. Hartl, *Cell* **83**, 589–598 (1995).
24. S. Takayama et al., *EMBO J.* **16**, 4887–4896 (1997).
25. D. Bimston et al., *EMBO J.* **17**, 6871–6878 (1998).
26. E.A. Nollen et al., *Mol. Cell. Biol.* **20**, 1083–1088 (2000).
27. J. Luders, J. Demand, and J. Hohfeld *J. Biol. Chem.* **275**, 4613–4617 (2000).
28. J. Kim, C. Zwieb, C. Wu, and S. Adhya, *Gene* **85**, 15–23 (1989).
29. E.A. Craig, J.S. Weissman, and A.L. Horwich, *Cell* **78**, 365–372 (1994).
30. T. Langer and W. Neupert, in *The Biology of Heat Shock Proteins and Molecular Chaperones*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1994, pp. 53–84.

31. B. Bercovich et al., *J. Biol. Chem.* **272**, 9002–9010 (1997).
32. W. Wagner and G. Kollias, *Urologe-Ausgabe A* **14**, 213–216 (1975).
33. R.N. Johnston and B.L. Kucey, *Science* **242**, 1551–1554 (1988).
34. K.T. Riabowol, L.A. Mizzen, and W.J. Welch, *Science* **242**, 433–436 (1988).
35. G.C. Li et al., *Proc. Natl. Acad. Sci. U.S.A.* **89**, 2036–2040 (1992).
36. R.Y. Liu, X. Li, L. Li, and G.C. Li, *Cancer Res.* **52**, 3667–3673 (1992).
37. C.R. Brown, L.Q. Hong-Brown, S.J. Doxsey, and W.J. Welch *J. Biol. Chem.* **271**, 833–840 (1996).
38. I.J. Benjamin and R.S. Williams, in *The Biology of Heat Shock Proteins and Molecular Chaperones*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1994, pp. 533–552.
39. U. Feige, R.I. Morimoto, I. Yahara, and B.S. Polla, in *Stress-Inducible Cell Responses* (1997).
40. U. Feige and W. Van Eden, in *Stress-Inducible Cellular Responses*, Birkhauser-Verlag, Basel-Boston-Berlin, 1997, pp. 359–374.
41. T.S. Nowak and H. Abe, in *The Biology of Heat Shock Proteins and Molecular Chaperones*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1994.
42. R. Mestril et al., *J. Clin. Invest.* **93**, 759–767 (1994).
43. M.S. Marber et al., *J. Clin. Invest.* **95**, 1446–1456 (1995).
44. J.C. Plumier et al., *J. Clin. Invest.* **95**, 1854–1860 (1995).
45. D.R. Ciocca et al., *J. Natl. Cancer Inst.* **85**, 570–574 (1993).
46. L.M. Vargas-Roig et al., *Int. J. Cancer* **79**, 468–475 (1998).
47. A. Jameel et al., *Int. J. Cancer* **50**, 409–415 (1992).
48. Y. Yufu, Y. Okada, T. Goto, and J. Nishimura, *Jpn. J. Clin. Oncol.* **22**, 411–413 (1992).
49. P.L. Hsu and S.M. Hsu, *Cancer Res.* **58**, 5507–5513 (1998).
50. C. Garrido et al., *Cancer Res.* **58**, 5495–5499 (1998).
51. M. Jaattela, and D. Wissing, *Ann. Med.* **24**, 249–258 (1992).
52. W. Voos et al., *J. Cell Biol.* **123**, 119–126 (1993).
53. J. Nylandsted et al., *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7871–7876 (2000).
54. D.P. Lane, C. Midgley, and T. Hupp, *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **339**, 369–372, discussion 372–3. (1993).
55. A. Ziemiecki, M.G. Catelli, I. Joab, and B. Moncharmont, *Biochem. Biophys. Res. Commun.* **138**, 1298–1307 (1986).
56. Y. Xu, M.A. Singer, and S. Lindquist, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 109–114 (1999).
57. L. Whitesell, S.D. Shifrin, G. Schwab, and L.M. Neckers, *Cancer Res.* **52**, 1721–1728 (1992).
58. Y. Xu and S. Lindquist, *Proc. Nat. Acad. Sci. U.S.A.* **90**, 7074–7078 (1993).
59. O. Pinhasi-Kimhi, D. Michalovitz, A. Ben-Zeev, and M. Oren, *Nature* **320**, 182–184 (1986).
60. E.T. Sawai and J.S. Butel, *J. Virol.* **63**, 3961–3973 (1989).
61. P.J. Koskinen et al., *J. Virol.* **65**, 842–851 (1991).
62. C.A. Finlay et al., *Mol. Cell. Biol.* **8**, 531–539 (1988).
63. J. Song, M. Takeda, and R.I. Morimoto, *Nat. Cell Biol.* **3**, 276–282 (2001).
64. D.D. Mosser et al., *Mol. Cell. Biol.* **17**, 5317–5327 (1997).
65. K.A. Buzzard, A.J. Giaccia, M. Killender, and R.L. Anderson, *J. Biol. Chem.* **273**, 17147–17153 (1998).
66. P. Pandey et al., *EMBO J.* **19**, 4310–4322 (2000).
67. H.M. Beere, R.I. Morimoto, and J.A. Hickman, *Cancer Res.* **53**, 3034–3039 (1993).
68. V.L. Gabai et al., *J. Biol. Chem.* **272**, 18033–18037 (1997).
69. M. Jaattela et al., *EMBO J.* **17**, 6124–6134 (1998).
70. E.H. Richards, E. Hickey, L. Weber, and J.R. Master, *Cancer Res.* **56**, 2446–2451 (1996).
71. F. Trautinger et al., *J. Photochem. Photobiol. B.* **39**, 90–95 (1997).
72. G. Multhoff et al., *Int. J. Cancer* **61**, 272–279 (1995).
73. A. Altmeyer et al., *Int. J. Cancer* **69**, 340–349 (1996).
74. R. Suto and P.K. Srivastava, *Science* **269**, 1585–1588 (1995).
75. P.K. Srivastava and R.G. Maki, *Curr. Top. Microbiol. Immunol.* **167**, 109–123 (1991).
76. P.K. Srivastava, *Experientia* **50**, 1054–1060 (1994).
77. A. Kakizuka, *Trends Genet.* **14**, 396–402 (1998).
78. G.S. Jackson et al., *Science* **283**, 1935–1937 (1999).
79. J.L. Jimenez et al., *EMBO J.* **18**, 815–821 (1999).
80. E. Scherzinger et al., *Cell* **90**, 549–558 (1997).
81. Y.O. Chernoff et al., *Science* **268**, 880–884 (1995).
82. E.C. Schirmer and S. Lindquist, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13932–13937 (1997).
83. S.H. Satyal et al., *Proc. Natl. Acad. Sci. U.S.A.* **97**, 5750–5755 (2000).
84. A. Wyttenbach et al., *Proc. Natl. Acad. Sci. U.S.A.* **97**, 2898–2903 (2000).
85. C.J. Cummings et al., *Nat. Genet.* **19**, 148–154 (1998).
86. S. Krobitsch and S. Lindquist, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 1589–1594 (2000).
87. J.M. Warrick et al., *Nat. Genet.* **23**, 425–458 (1999).
88. P. Kazemi-Esfarjani and S. Benzer, *Science* **287**, 1837–1840 (2000).
89. J. Lis and C. Wu, *Cell* **74**, 1–4 (1993).
90. R.I. Morimoto, *Science* **259**, 1409–1410 (1993).
91. C. Wu, *Annu. Rev. Cell Develop. Biol.* **11**, 441–469 (1995).
92. R.I. Morimoto and M.G. Santoro, *Nat. Biotechnol.* **16**, 833–838 (1998).
93. G. Wiederrecht, D. Seto, and C.S. Parker, *Cell* **54**, 841–853 (1988).
94. P.K. Sorger and H.R. Pelham, *Cell* **54**, 855–864 (1988).
95. J. Clos et al., *Cell* **63**, 1085–1097 (1990).
96. S.K. Rabindran, G. Giorgi, J. Clos, and C. Wu, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6906–6910 (1991).
97. K.D. Sarge et al., *Genes Dev.* **5**, 1902–1911 (1991).
98. A. Nakai and R.I. Morimoto, *Mol. Cell. Biol.* **13**, 1983–1997 (1993).
99. A. Nakai et al., *Mol. Cell. Biol.* **17**, 469–481 (1997).
100. T.J. Schuetz et al., *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6911–6915 (1991).
101. C.J. Harrison, A.A. Bohm, and H.C. Nelson, *Science* **263**, 224–227 (1994).
102. G.W. Vuister et al., *Nat. Struct. Biol.* **1**, 605–614 (1994).
103. P.K. Sorger and H.C. Nelson, *Cell* **59**, 807–813 (1989).
104. R. Peteranderl and H.C. Nelson, *Biochemistry* **31**, 12272–12276 (1992).
105. M. Green, T.J. Schuetz, E.K. Sullivan, and R.E. Kingston, *Mol. Cell. Biol.* **15**, 3354–3362 (1995).
106. Y. Shi, P.E. Kroeger, and R.I. Morimotor, *Mol. Cell. Biol.* **15**, 4309–4318 (1995).
107. J. Zuo, D. Rungger, and R. Voellmy, *Mol. Cell. Biol.* **15**, 4319–4330 (1995).
108. J. Wisniewski, A. Orosz, R. Allada, and C. Wu, *Nucleic Acids Res.* **24**, 367–374 (1996).
109. T. Farkas, Y.A. Kutskova, and V. Zimarino *Mol. Cell. Biol.* **18**, 906–918 (1998).
110. S.K. Rabindran et al., *Science* **259**, 230–234 (1993).

111. J. Zuo, R. Baler, G. Dahl, and R. Voellmy, *Mol. Cell. Biol.* **14**, 7557–7568 (1994).
112. A. Orosz, J. Wisniewski, and C. Wu, *Mol. Cell. Biol.* **16**, 7018–7030 (1996).
113. E. Zandi, T.N. Tran, W. Chamberlain, and C.S. Parker *Genes Dev.* **11**, 1299–1314 (1997).
114. R. Baler, G. Dahl, and R. Voellmy, *Mol. Cell. Biol.* **13**, 2486–2496 (1993).
115. K. D. Sarge, S.P. Murphy, and R.I. Morimoto, *Mol. Cell. Biol.* **13**, 1392–1407 (1993).
116. A. Nakai et al., *Mol. Cell. Biol.* **15**, 5268–5278 (1995).
117. M. Tanabe, A. Nakai, Y. Kawazoe, and K. Nagata, *J. Biol. Chem.* **272**, 15389–15395 (1997).
118. M. Tanabe et al., *EMBO J.* **17**, 1750–1758.
119. C. Kanei-Ishii et al., *Science* **277**, 246–248 (1997).
120. C.M. Cahill et al., *Adv. Exp. Med. Biol.* 625–630 (1997).
121. N.G. Theodorakis et al., *Mol. Cell. Biol.* **9**, 3166–3173 (1989).
122. L. Sistonen et al., *Mol. Cell. Biol.* **12**, 4104–4111 (1992).
123. L. Sistonen, K.D. Sarge, and R.I. Morimoto, *Mol. Cell. Biol.* **14**, 2087–2099 (1994).
124. K.D. Sarge et al., *Biol. Reprod.* **50**, 1334–1343 (1994).
125. V. Mezger et al., *Develop. Biol.* **166**, 819–822 (1994).
126. M. Rallu et al., *Proc. Natl. Acad. Sci. U.S.A.* **94**, 2392–2397 (1997).
127. A. Mathew, S.K. Mathur, and R.I. Morimoto, *Mol. Cell. Biol.* **18**, 5091–5098 (1998).
128. R.I. Morimoto, *Genes Dev.* **12**, 3788–3796 (1998).
129. J.S. Larson, T.J. Schuetz, and R.E. Kingston, *Nature* **335**, 372–375 (1988).
130. D.D. Mosser, P.T. Kotzbauer, K.D. Sarge, and R.I. Morimoto, *Proc. Natl. Acad. Sci. U.S.A* **87**, 3748–3752 (1990).
131. V. Zimarino, C. Tsai, and C. Wu, *Mol. Cell. Biol.* **10**, 752–759 (1990).
132. J.T. Westwood, J. Clos, and C. Wu, *Nature* **353**, 822–827 (1991).
133. U. Knauf, E.M. Newton, J. Kyriakis, and R.E. Kingston, *Genes Dev.* **10**, 2782–2793 (1996).
134. M.P. Kline and R.I. Morimoto, *Mol. Cell. Biol.* **17**, 2107–2115 (1997).
135. D.A. Jurivich, L. Sistonen, R.A. Kroes, and R.I. Morimoto, *Science* **255**, 1243–1245 (1992).
136. C. Giardina and J.T. Lis, *J. Biol. Chem.* **270**, 10369–10372 (1995).
137. J.J. Cotto, M. Kline, and R.I. Morimoto, *J. Biol. Chem.* **271**, 3355–3358 (1996).
138. B.J. DiDomenico, G.E. Bugaisky, and S. Lindquist, *Cell* **31**, 593–603 (1982).
139. R.P. Beckmann, L.E. Mizzen, and W.J. Welch, *Science* **248**, 850–854 (1990).
140. D.E. Stone and E.A. Craig, *Mol. Cell. Biol.* **10**, 1622–1632 (1990).
141. W.R. Boorstein and E.A. Craig, *Mol. Cell. Biol.* **10**, 3262–3267 (1990).
142. R.J. Nelson, M.F. Heschl, and E.A. Craig, *Genetics* **131**, 277–285 (1992).
143. J.T. Halladay and E.A. Craig, *Mol. Cell. Biol.* **15**, 4890–4897 (1995).
144. K. Abravaya, M.P. Myers, S.P. Murphy, and R.I. Morimoto, *Genes Dev.* **6**, 1153–1164 (1992).
145. R. Baler, W.J. Welch, and R. Voellmy, *J. Cell Biol.* **117**, 1151–1159 (1992).
146. S.K. Rabindran et al., *Mol. Cell. Biol.* **14**, 6552–6560 (1994).
147. S.L. Nunes and S.K. Calderwood, *Biochem. Biophys. Res. Com.* **213**, 1–6 (1995).
148. Y. Shi, D.D. Mosser, and R.I. Morimoto, *Genes Dev.* **12**, 654–666 (1998).
149. G. Marchler and C. Wu, *EMBO J.* **20**, 499–509 (2001).
150. M. Zhong et al., *Methods Enzymol.* **274**, 113–119 (1996).
151. K. Nadeau, A. Das, and C.T. Walsh, *J. Biol. Chem.* **268**, 1479–1487 (1993).
152. L. Vigh et al., *Nat. Med.* **3**, 1150–1154 (1997).
153. B.S. Lee et al., *Proc. Natl. Acad. Sci. U.S.A.* **92**, 7207–7211 (1995).
154. C. Amici, L. Sistonen, M.G. Santoro, and R.I. Morimoto, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 6227–6231 (1992).
155. A. Rossi, G. Elia, and M.G. Santoro, *J. Biol. Chem.* **273**, 16446–16452 (1998).
156. L.H. Li, T.D. Clark, C.H. Cowie, and K.L. Rinehart, Jr., *Cancer Treat. Rep.* **61**, 815–824 (1977).
157. Y. Uehara, M. Hori, T. Takeuchi, and H. Umezawa, *Mol. Cell. Biol.* **6**, 2198–2206 (1986).
158. C. Prodromou et al., *Cell* **90**, 65–75 (1997).
159. C.E. Stebbins et al., *Cell* **89**, 239–250 (1997).
160. L. Whitesell et al., *Proc. Natl. Acad. Sci. U.S.A.* **91**, 8324–8328 (1994).
161. D.F. Smith et al., *Mol. Cell. Biol.* **15**, 6804–6812 (1995).
162. L. Whitesell and P. Cook, *Mol. Endocrinol.* **10**, 705–712 (1996).
163. S.V. Sharma, T. Agatsuma, and H. Nakano *Oncogene* **16**, 2639–2645 (1998).

HELICASES

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Helicases are enzymes that unwind DNA or RNA helices. They participate in central biosynthetic processes, including DNA replication, recombination, repair, and transcription, as well as RNA maturation and translation. Almost all pathways that alter nucleic acid structures require the action of a helicase. Those unwinding DNA duplexes are classified as DNA helicases. Those unwinding double-stranded RNA are designated as RNA helicases. Some helicases also possess activities that can unravel RNA-DNA hybrids. The importance of helicases in human cellular processes is signified by the fact that mutations in different DNA helicases in humans result in specific genetic disorders (1,2).

CONSERVED AMINO ACID MOTIFS

There are multiple helicases in prokaryotic and eukaryotic cells. Presumably related to their biological functions, helicases are evolutionarily conserved; most of them contain a domain ranging from 300 to 500 amino acids that encompasses seven characteristic motifs. The seven amino acid motifs are designated I, Ia, and II–VI, where I and II (also called Walker box A and B, respectively) comprise the ATPase domain responsible for the binding and hydrolysis of nucleotide triphosphates (Fig. 1). DNA helicases normally contain a DEXH box [Asp-Glu-any amino acid-His (aspartic acid-glutamic acid-any amino acid-histidine)] in motif II, whereas RNA helicases contain either a DEXH box or a DEAD (Asp-Glu-Ala-Asp) box (Fig. 1).