REVIEWS

Is hsp70 the cellular thermometer?

Elizabeth A. Craig and Carol A. Gross

Cells respond to an increase in temperature by inducing the synthesis of the heat shock proteins, which are a small set of evolutionarily conserved proteins. We review the evidence leading us to suggest that the free pool of one of these proteins, hsp70, serves as a cellular thermometer that regulates the expression of all heat shock proteins.

THE HEAT SHOCK RESPONSE occurs when cells growing at a low temperature are shifted to a higher temperature, either one that still permits growth or a lethal temperature, and results in the induction of a small set of proteins called the heat shock proteins or hsps (for review see Ref. 1). Synthesis of this class of proteins, in contrast to most proteins, increases upon temperature upshift and decreases upon downshift. This response to temperature is nearly universal among living organisms including both those that live in low temperature environments and those from thermal springs. Not only is the response to temperature conserved, but the temperature-inducible proteins are conserved as well. Recent evidence suggests that the way in which all cells sense changes in temperature may also be conserved. In this article, we compare the prokaryotic and eukaryotic heat shock response, describe the functions of the 70 kDa heat shock protein hsp70 and present the evidence that leads us to the view that hsp70 is the cellular thermometer.

The heat shock response

The molecular response to temperature upshift has been extensively studied in both prokaryotic and eukaryotic organisms. An example of the heat shock response in *E. coli* and in cultured *Drosophila* cells is shown in Fig. 1. In both cases, cells respond to temperature upshift by increasing either the amount or the activity of a transcription factor that is specific for the heat shock genes. The result is increased transcription of the heat shock genes, which leads to an increase in the concentration of hsps in the cell. The magnitude of the response and its duration depend on the severity of the temperature upshift. The maximal response is obtained after a shift to a lethal temperature, in which case, non-hsp synthesis is shut off and cells make hsps at maximal rates as long as protein synthesis continues. In addition to heat, other stresses such as ethanol, virus infection, amino acid analogues and DNA damage can alter expression of the heat shock proteins. In cases where mechanisms have been addressed, these stresses also function by increasing the amount or activity of the heat shock transcription factor. A converse response, that is, a selective reduction in hsp synthesis, is seen when cells are shifted down in temperature.

Prokaryotic organisms. Among prokaryotic organisms, the heat shock response has been most intensively studied in *E. coli*. In this organism, an alternative sigma factor functions as the heat-shock-specific transcription factor (for review see Ref. 2). At normal growth temperatures, most transcription in the cell is carried out by RNA polymerase holoenzyme (E), which contains the housekeeping 70 kDa sigma factor (σ^{70}). Heat shock promoters are not

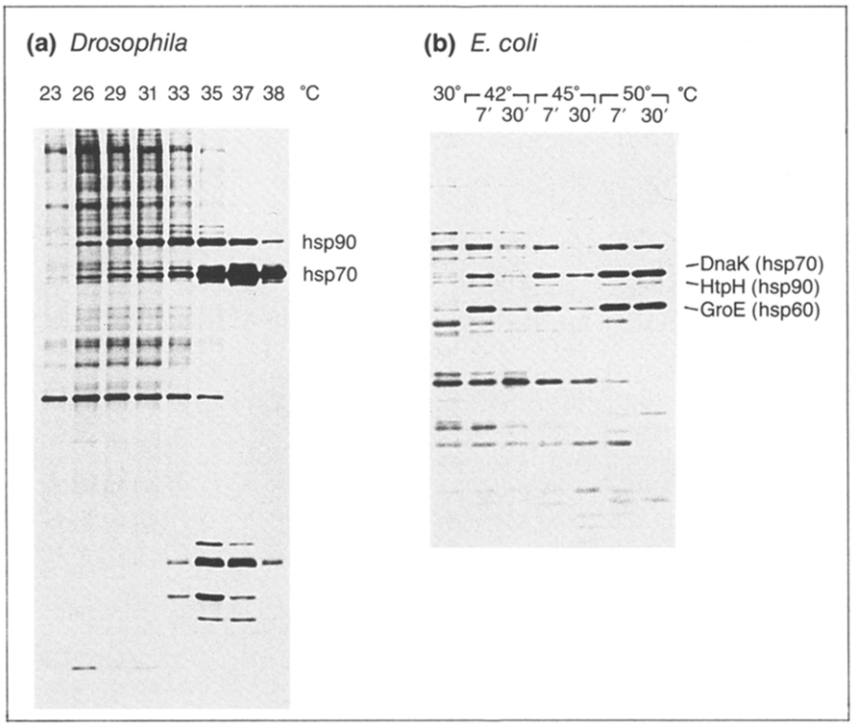

Figure 1
(a) Cultured *Drosophila* cells growing at 23°C were shifted to the temperature indicated for 60 minutes and labeled with [^{35}S]methionine. **(b)** *E. coli* cells growing at 30°C were shifted to the indicated temperatures and labeled with [^{35}S]methionine at either 7 minutes or 30 minutes after temperature upshift.

E. A. Craig is at the Department of Physiological Chemistry and **C. A. Gross** is at the Department of Bacteriology, University of Wisconsin–Madison, Madison, WI 53706, USA.

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recognized by the holoenzyme $E\sigma^{70}$, but instead by one containing a 32 kDa sigma factor (σ^{32}). In addition to directing the transcription apparatus to heat shock promoters, σ^{32} also functions as the direct regulator of the amount of heat shock gene transcription following temperature shift.

Following temperature upshift, a transient increase in the cellular concentration of σ^{32} leads to a transient increase in the rate of hsp synthesis. During steady-state growth at low temperature (30°C), there are only about 10–30 molecules of σ^{32} in the cell. Two regulatory mechanisms function together to keep the amount of σ^{32} at a low level. First, σ^{32}, with a half-life of only one minute, is among the most unstable proteins known in *E. coli*. Second, expression of σ^{32} is repressed at the translational level. Following temperature upshift, both the stability and synthesis of σ^{32} transiently increase, resulting in a corresponding increase in the concentration of σ^{32}. Because σ^{32} is present in limiting amounts in the cell, an increase in the concentration of σ^{32} is followed by a proportionate increase in the transcription of heat shock genes, resulting in a transient increase in the rate of hsp synthesis.

Following temperature downshift, the converse effect is seen. The rate of transcription initiation at heat shock promoters, and consequently the rate of synthesis of hsps, transiently decreases[3,4]. However, in contrast to the situation upon upshift, repression of hsp transcription is not mediated by a drop in the concentration of σ^{32}. Instead, σ^{32} appears to be inactivated upon temperature downshift.

Eukaryotic organisms. The regulation of transcription after a heat shock has been studied in a variety of eukaryotes, in particular *Saccharomyces*, *Drosophila* and mammalian cells. A similar mechanism is responsible for regulation in these diverse organisms (for review see Ref. 5). A positively acting transcription factor, termed heat shock factor (HSF), binds to a promoter element, termed a heat shock element (HSE), facilitating transcription. The HSE, like many enhancer-like sequences, is active at variable distances from the start site of transcription. However, both HSEs and HSF have unusual characteristics.

A functional HSE is modular, consisting of a variable number of 5-bp units called 'GAA boxes' (i.e. the sequence *n*GAA*n*). These boxes may have either a head-to-head (GAA-TTC) or tail-to-tail (TTC-GAA) arrangement[6,7]. Extended HSEs, containing up to seven *n*GAA*n* units, are present in some heat shock promoters, although in some cases, *n*GAA*n* units may be separated by 5 bp of unrelated sequence. Data from both yeast and *Drosophila* indicate that HSF is trimeric[8,9], with each monomer interacting with a 'GAA' box. While the deduced protein sequences from *Drosophila*, *Saccharomyces* and *Kluyveromyces* show little overall conservation, the DNA-binding domain has been conserved (C. Wu and H. Pelham, pers. commun.), which is consistent with experiments indicating interactions of HSF with HSEs are similar among divergent species.

Among the HSFs studied, the yeast factor is unique in its ability to bind DNA under non-stress conditions[10]. In fact, yeast HSF is essential under all growth conditions and is necessary for much of the high constitutive expression of several heat shock genes under optimal growth conditions. In cells from other organisms, such as *Drosophila* and mammals, both the DNA binding and transcriptional activities of HSF are induced by heat shock. Since DNA-binding activity has been induced *in vitro* by changes in pH, increases in temperature and interaction with specific antibodies, it has been suggested that a conformational change is responsible for the gain in DNA-binding activity[11,12].

Increased phosphorylation of HSF is correlated with an increased ability to promote transcription in all eukaryotes studied so far. It could be that this phosphorylation is directly responsible for the activation of HSF, perhaps by creating an 'acid blob', a term for a domain present in several transcription factors known to be responsible for transcriptional activation. However, this simple explanation may not be correct. An analysis of deletion mutants of HSF suggests that the activity of a cryptic constitutive activator is repressed in the absence of heat shock by adjacent regions of the protein[13,14]. Perhaps phosphorylation serves to maintain a conformational change in HSF, thereby unmasking this activator domain.

In conclusion, the temperature-dependent increase in hsp expression in both prokaryotes and eukaryotes is effected through positively acting transcription factors. The activity of the transcriptional activator itself, however, is regulated by a complex series of post-transcriptional controls. In *E. coli*, the rate of synthesis, stability and activity of σ^{32} can all be altered to adjust the rate of transcription of heat shock genes. In eukaryotes, the activity of HSF is profoundly affected by increased temperature, or by the presence of other inducers. Interestingly, a general pattern of post-transcriptional control of the transcriptional regulators governing expression of sets of genes in response to environmental conditions seems to be emerging. The control circuits governing the lysis–lysogeny decision in bacteriophage λ, gene expression after UV irradiation and the expression of genes regulated by the two-component sensory systems are all based on transcriptional factors whose activity is controlled post-transcriptionally. This arrangement may facilitate a very rapid response to a change in environmental conditions.

Structure and function of hsp70

The hsps70, which are found in both eukaryotes and prokaryotes, are among the most highly conserved hsps. Recent work suggests that hsps70 play a central but previously unsuspected role in normal cell growth, which involves binding to and release from other polypeptides to facilitate (or prevent) inter- and intra-molecular interactions. The current thought is that they perform the same function after exposure to stress conditions. Each inducing condition would be expected to create excess substrates, thus necessitating a greater abundance of hsps. This view suggests that these proteins are essential for cell viability even under non-stress conditions, but that their expression is highly regulated to achieve the appropriate concentration under inducing conditions.

In most organisms, hsp70 is the most abundant heat shock protein. In *E. coli* (and presumably other prokaryotes as well) there is only a single hsp70-related protein, the product of the *dnaK* gene, which is essential for growth (for review see Ref. 1). However, in most if not all eukaryotes, there are multiple genes encoding a set of related hsp70 proteins, some of which are present under optimal growth conditions, while others are expressed after a stress. These proteins are found in several compartments of the cell. The major inducible member is usually found predominantly in the nucleus during and immediately after stress, moving to the cytoplasm upon recovery. Two constitutively expressed pro-

teins are present in organelles, one in the endoplasmic reticulum (ER) and the other in the mitochondrion. Genetic analysis in yeast has shown that these three classes of hsps70 perform essential functions in their respective compartments.

Biochemical characterization of hsps70 has uncovered a number of shared properties. All hsps70 examined bind ATP with high affinity and possess a weak ATPase activity (for review see Ref. 1). *In vitro* studies with mammalian proteins have shown that hsps70 can interact with a variety of peptides, and that their release is dependent upon the hydrolysis of ATP[15]. As discussed below, hsps70 have been shown to participate in a number of specific protein–protein interactions. In the current, unifying view, hsps70 bind to protein substrates, altering or maintaining conformations or interactions with other proteins; release from such substrates is ATP-dependent (Fig. 2).

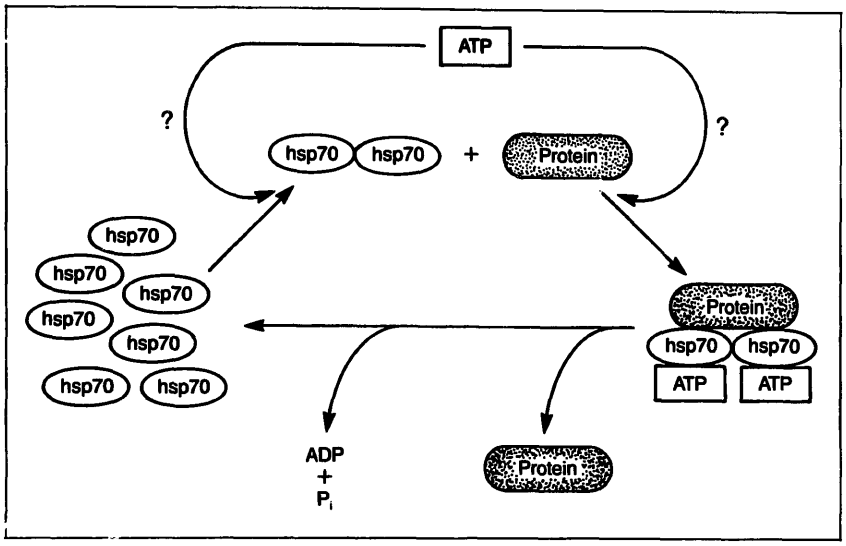

Figure 2

This cartoon illustrates the current paradigm for hsp70 action and also indicates the current uncertainties about its mode of action. While hsp70 is purified predominantly as a monomer, the functional form of hsp70 is believed to be a multimer of unknown size. We indicate this uncertainty by showing a monomer to dimer step in the cycle. ATP facilitates hsp70 binding to substrate in some systems (clathrin) and the release from substrate (synthetic peptides) in others. We indicate this by showing that ATP could be involved at two different steps in the process. Finally, no role for the auxilliary DnaJ and GrpE proteins is indicated.

The hsp70 proteins have been highly conserved, showing 60–78% identity among eukaryotic proteins and 40–60% identity between the *E. coli* hsp70, DnaK, and the eukaryotic proteins (for review see Ref. 16). While regions of identity extend over large portions of the proteins, in general the amino-terminal two-thirds of the protein is much more highly conserved than the carboxy-terminal portion. There are regions of particularly striking identity within this amino-terminal portion. The ATP-binding domain and the ATPase activity are associated with the 44 kDa terminal fragment[17], suggesting that the less highly conserved carboxy-terminal portion may have evolved to interact with a particular set of protein substrates, while the more conserved regions are associated with biochemical properties common to all hsps70.

The first hsp70-like gene to be studied was *dnaK*, which was initially identified along with *dnaJ* and *grpE* as *E. coli* genes required for the growth of bacteriophage λ (Ref. 18). The DnaK, DnaJ and GrpE proteins encoded by these genes function together in the sequential multi-protein pathway that results in activation of the λ origin of replication (oriλ). The initial nucleoprotein structure formed at the origin is partially disassembled by DnaK and DnaJ in an ATP-dependent manner, thus allowing replication to proceed (for review see Ref. 19). Subsequent investigations have indicated important roles for DnaK in the normal growth of *E. coli*, although the specifics of such involvements have been difficult to determine. Mutations in *dnaK* seem to affect DNA synthesis, RNA synthesis and cell division (for review see Ref. 19) as well as export of certain fusion proteins[20]. Recent *in vitro* studies indicate that DnaK can interact with denatured protein, promoting renaturation. Specifically, interactions with RNA polymerase[21] and λ repressor[22] have been reported. Interestingly, DnaK also promotes renaturation of temperature-sensitive λ repressor *in vivo*[22].

Because of their close relationship with DnaK, the DnaJ and GrpE proteins, which are also hsps, deserve some comment. A considerable body of evidence indicates that they modulate the activity of DnaK. There is biochemical evidence for the interaction of these proteins[22]. Mutations in each of these genes have very similar effects on cell physiology, suggesting the participation of all three proteins in the same processes. It has already been established that eukaryotic homologs of DnaJ exist[23]. Hybridization experiments indicate that eukaryotic organisms contain sequences that cross react with GrpE as well, suggesting that the functional interactions between these three proteins observed in *E. coli* will also be true in eukaryotic cells (for review see Ref. 19). These studies also introduce a note of caution for *in vitro* studies examining the function of hsp70 in the absence of additional cellular factors that may modulate its activity.

The vast majority of the data concerning eukaryotic hsp70 function also support the idea that these proteins interact with a variety of cellular proteins, and are essential for the movement of proteins within cells, from the time they are synthesized until they have reached their final destination, be it transport through the secretory pathway[24], translocation into mitochondria[25,26] or degradation in the lysosome under some starvation conditions[27]. The cytoplasmic hsps70 that have been implicated in the regulation of the heat shock response have been shown to be involved in a variety of cellular processes. The first hsp70 to be associated with a biochemical activity was the protein purified because of its ability to release clathrin from coated vesicles that mediate endocytosis (for review see Ref. 26). The uncoating enzyme (hsc70), later shown to be an hsp70, hydrolysed ATP in a clathrin-dependent manner, driving disassembly of the clathrin coat. Genetic and biochemical evidence demonstrated a role in protein translocation for hsps70 localized to the cytoplasm. It is likely that hsp70 is required to maintain precursors in a translocation-competent conformation.

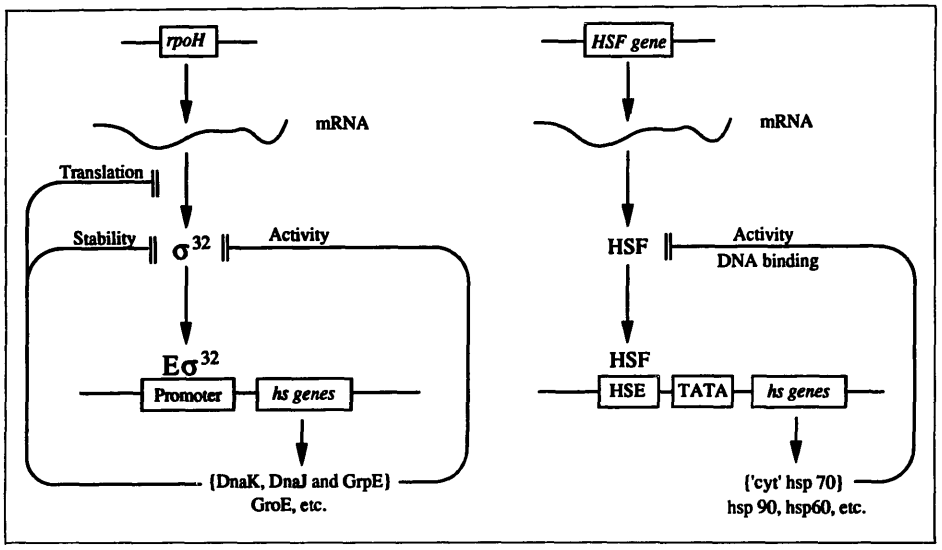

Figure 3

A speculative model for the role of hsp70 (DnaK) in controlling expression of hsps, showing how the cytoplasmic hsp70 (DnaK), along with DnaJ and GrpE (and eukaryotic homologs), could act to control hsp expression in both prokaryotes and eukaryotes. Upon temperature upshifts, depletion of the free pool of these hsps relieves their negative regulatory effects. Increased synthesis and stability of σ^{32} and increased activity of HSF permit increased transcription of heat shock genes. Note that in all eukaryotic organisms, except for *S. cerevisiae*, stress induces both DNA binding and activity of HSF. In *S. cerevisiae*, DNA binding is constitutive and stress serves solely to convert HSF to the activator form.

In addition, cytoplasmic hsp70 appears to interact with many newly synthesized proteins, since many pulse-labeled proteins of human cells co-immunoprecipitate with hsp70 antibody[28]. In conclusion, the underlying theme of the biological processes in which hsp70 is involved is protein–protein interactions. This role for hsp70 is a critical part of the model of regulation of the heat shock response discussed in the next section.

The cellular thermometer

How do cells sense changes in temperature? While the answer to this question is presently unknown, recent evidence suggests that a homeostatic mechanism involving the level of free hsp70 in the cell provides a thermometer for reacting to temperature changes. This general idea was first proposed by S. Lindquist, based on the analysis of the response of *Drosophila* cells to temperature and amino acid analogs[29]. We will summarize the evidence that supports such a model.

In both eukaryotes and prokaryotes, mutations in hsp70 result in an increased expression of hsps at optimal growth temperatures. In yeast, strains carrying mutations in the two constitutively expressed genes that encode cytoplasmic hsp70 (*SSA1* and *SSA2*), express hsps at high levels even at 23°C[30]. In addition, these strains are strikingly thermo-tolerant, a hallmark of expression of heat shock proteins. In *E. coli*, mutations in *dnaK* result in increased expression of hsps at 30°C, prolong the high rate of expression of hsps after shift to high temperature and abolish the decrease in hsp expression normally seen after shift to low temperature[3,31]. The fact that loss of function mutations in *dnaK* lead to increased hsp expression suggests that the function of the wild-type DnaK protein is to act as a negative regulator of the expression of hsps. Mutations in *dnaJ* and *grpE* have the same heat shock phenotypes as those in *dnaK*, suggesting that the DnaK, DnaJ and GrpE proteins function together as negative regulators of the expression of hsps[32].

In both yeast and *E. coli*, mutations in hsp70 appear to promote expression of hsps by working through the transcription factor responsible for regulating inducible transcription of heat shock genes. In yeast, the target of HSP70 regulation appears to be HSF, since constitutive expression of hsps in yeast strains carrying mutations in *SSA1* and *SSA2* can be eliminated by mutating HSEs, the binding sites for HSF. In addition, HSEs fused to heterologous promoters drive constitutive expression in strains that lack the constitutively expressed hsp70 genes, but not in wild-type cells[33]. In *E. coli*, the target of DnaK, DnaJ and GrpE regulation is σ^{32}, the transcription factor that is the direct regulator of the heat shock response. These proteins negatively regulate σ^{32} in three ways: they are required for translational regulation of σ^{32} synthesis at high temperature, for inactivation of σ^{32} after shift to low temperature and they facilitate σ^{32} degradation at all temperatures[32,34].

The findings that DnaK, DnaJ and GrpE are involved in every process known to regulate σ^{32} and that hsp70 mutations result in activation of HSF suggest cells might use the functional state of these proteins to sense temperature change. In such a model, a temperature increase generates an increase in the concentration of 'substrates', which temporarily depletes the free pool of hsp70 (and DnaJ, GrpE and their eukaryotic homologs), while a temperature decrease would result in decreased substrates, temporarily expanding the free pool of these proteins. These 'substrates' would include not only unstable and pre-existing proteins whose conformation may be altered by temperature shift, but also (and probably more importantly) products of normal cellular metabolism such as nascent proteins and translocation-competent precursors, which interact with hsp70. Performing a temperature upshift would be functionally equivalent to introducing an hsp70 (*dnaJ* or *grpE*) mutation because it would temporarily interfere with the ability of the cell to regulate the activity level of HSF or degrade and repress the synthesis of σ^{32}. This response would be self-limiting because the ensuing overproduction of hsps would restore the free pool of these hsps to appropriate levels to re-establish appropriate regulation of σ^{32} or HSF. Conversely, a temperature downshift would be functionally equivalent to overexpressing these hsps.

If temperature is sensed by a homeostatic mechanism linking the function of hsp70 (and DnaJ and GrpE) to the regulation of σ^{32} or HSF, then the following

must be true: (1) free hsp70 must be present in limiting concentrations in the cell, (2) the demand for these proteins must increase with temperature and (3) production of excess substrates of hsp70 should induce the heat shock response without temperature shift. The first two postulates have been shown to be true in both eukaryotes and prokaryotes. In *E. coli* this was demonstrated in a strain in which the rate of hsp synthesis can be varied by manipulating the amount of σ^{32} (Ref. 35). The fact that the maximum growth temperature of this strain is determined by the cellular capacity to make heat shock proteins indicates both that heat shock proteins are limiting and that more hsps are required at high temperature. Comparable experiments in eukaryotic cells rely on mutants of yeast that contain a partial complement of the hsp70 genes encoding cytoplasmic hsp70. Such cells grow slowly at low temperatures and are temperature sensitive for growth. The fact that both of these phenotypes are relieved by increasing the expression of the remaining genes again indicates that hsps are limiting and that there is increased demand for these proteins at higher temperatures (E. Craig, unpublished).

That production of excess substrates of hsp70 results in the induction of the heat shock response in both eukaryotes and prokaryotes is suggested by the fact that a number of inducers of the heat shock response are likely to function by doing just this. Denatured λ repressor, which is a substrate for DnaK *in vitro*, has been shown to induce the heat shock response in *E. coli*[22,36]; injection of denatured proteins into *Xenopus* oocytes also induces the heat shock response[37]. Protein synthesis inhibitors that cause an accumulation of missense proteins, amino acid analogs or protein fragments induce hsps[29,38]. Also, the fact that puromycin induces the heat shock response is particularly informative, since there is good evidence that hsp70 is involved in the degradation of puromycyl-induced protein fragments. In eukaryotic cells, hsp70 has been found in permanent association with puromycyl fragments[28], while in prokaryotic cells, both mutant analysis and over-expression studies indicate that DnaK is involved in their degradation (for review see Ref. 2). Ethanol, an hsp inducer in most systems, may increase protein denaturation and/or increase the demand for hsps in protein folding. In addition, mutations other than those in hsp70 that result in the constitutive expression of hsps could work by causing the accumulation of hsp70 substrates. For example, disruption of genes for some ubiquitin-conjugating enzymes could induce hsps because the proteins, unable to be conjugated with ubiquitin, accumulate and bind hsp70 rather than entering the degradative pathway[39]. Likewise, a mutation in a gene essential for an early step in protein translocation across the ER membrane could cause hsp induction[40] because untranslocated precursors that bind hsp70 accumulate.

Taken together, the experiments described above suggest that raising the temperature would increase substrates of hsp70 and that depleting the free pool of hsp70 is sufficient to induce the heat shock response. While this analysis does not prove that a change in the free pool of this protein is the thermometer utilized by the cells, it certainly suggests that this hypothesis warrants serious attention.

If such a model were true, how would the hsp70 thermometer transduce the signal to activate HSF or increase the concentration of σ^{32}? The simplest model would have hsp70 interacting directly with HSF or σ^{32}. The possibility of such direct interactions are intriguing because they are consistent with the proposed function of hsp70 to interact with other proteins and maintain or alter their conformational states. For example, we have already described experiments suggesting that activation of HSF binding in higher eukaryotes may result from a conformational change, perhaps allowing trimerization. It may be that HSF is maintained in a monomeric state by virtue of its binding to hsp70. Upon heat shock, or in other situations where the pool of hsp70 is reduced, HSF would be released, resulting in formation of trimers capable of DNA binding. A parallel scenario would explain the effect of DnaK. In this case, binding of σ^{32} to DnaK would be required to allow rapid degradation by a yet to be identified proteolytic system. During steady-state growth, σ^{32} would be bound to DnaK, thus facilitating its degradation. Upon temperature upshift, reduction in the pool of free DnaK would release σ^{32}, preventing its degradation. Although the details of these hypothesized mechanisms in eukaryotes and prokaryotes differ, the central theme is the same – interaction of hsp70 with the factor that modulates transcription of the heat shock genes permits expression of the hsps to be keyed to the functional state of the cell. While intriguing, this model is clearly speculative. On the other hand, rank speculation will not last long, as we are confident that our colleagues will determine the true mechanism of hsp regulation in the near future.

Acknowledgements

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Molecular studies in Alzheimer's disease

David M. Holtzman and William C. Mobley

In the past decade, there has been an explosion of information relating to the molecular neurobiology of Alzheimer's disease (AD). Molecular dissection of the neuropathology of AD has provided insight into the pathogenesis of this disease and has defined areas where investigation may prove useful in elucidating the cause of this disorder and suggest new treatments.

OVER THE PAST TEN YEARS, Alzheimer's disease (AD) has been recognized as a major public health problem. Approximately 10% of the population over the age of 65 are affected by this form of progressive dementia. With the elderly population increasing, AD will have an even greater social and economic impact. Currently, there is no effective treatment for AD[1], so in order to develop effective therapies, it is essential that its causes be elucidated. The central nervous system (CNS) neuropathological and neurochemical hallmarks of AD include selective neuronal cell death, a decrease in markers for certain neurotransmitters and the presence of abnormal proteinaceous deposits in neurons (neurofibrillary tangles) as well as in the extracellular space (cerebrovascular, diffuse and neuritic plaques)[1-3]. Though none of these features is unique to AD, they are found in all cases of the disorder[1,2]. The occurrence of neurofibrillary tangles (NFTs), neuritic plaques and loss of certain neuronal populations are linked with dementia[1,2]. The present challenge is twofold: to determine how these hallmarks are linked to etiology and to determine whether their presence explains the symptoms of this disease. This review will focus on recent attempts to understand the molecular basis for AD and will address potential approaches for understanding its pathogenesis.

Amyloid precursor protein (APP) and the β/A4 protein

A key diagnostic feature of AD is the presence of neuritic and cerebrovascular plaques (Fig. 1). Neuritic plaques occur in the brain parenchyma and are spherical elements composed of dystrophic neuronal processes (neurites) intermingled with glial cells and filamentous proteinaceous deposits containing amyloid, as shown by thioflavin S and Congo red staining techniques. Amyloid deposits are also found in the walls of blood vessels in the brain and its covering membranes. Considerable effort has been directed at examining these lesions and their relationship to AD pathogenesis. Purification of both neuritic and cerebrovascular plaques has revealed that a principal component is a peptide of approximately 40–42 amino acids, which will be referred to as β/A4 (Refs 4,5). The use of immunohistochemistry has shown that β/A4 immunoreactivity occurs abundantly in the AD brain, not only in neuritic and cerebrovascular plaques, but also in the form of diffuse parenchymal CNS deposits (diffuse plaques) not associated with dystrophic neurites[3]. Using oligonucleotide probes based on the amino acid sequence of β/A4, it was shown that this peptide is encoded by a host gene for a much larger protein, which has been termed the amyloid precursor protein (APP)[6].

The discovery that β/A4 was encoded by *APP* encouraged studies to understand the normal structure and function of the precursor and the events leading to β/A4 deposition. *APP* has been localized to the long arm of human chromosome 21 (Ref. 6). It has been highly conserved through evolution and is expressed in a variety of tissues. APP mRNA levels are tissue specific and developmentally regulated. Several potential regulatory elements are found in the 5' flanking sequences of the gene[7]. Among other factors, nerve growth factor (NGF) and interleukin 1 have been shown to increase mRNA levels in responsive cell populations[8,9]. *APP* has at least 18 exons that are used to create five or more different APP transcripts through alternative splicing[10,11]. The predominant transcripts encode proteins of 695, 751 and 770 amino acids[6]. Organs with the highest levels of APP mRNA include brain, lung, kidney, muscle and spleen[12]. Interestingly, APP695 mRNA is enriched in mammalian brain, while the transcripts encoding APP751 and APP770 (APP751/770) predominate in other tissues[12]. The normal function of APP and the processes leading to cleavage of β/A4 from APP are under intense investigation. In several mammalian species, APP exists as a group of 110–135 kDa membrane-associated proteins[13,14]. APP

D. M. Holtzman and **W. C. Mobley** are at the Departments of Neurology, Pediatrics and the Neuroscience Program, University of California, San Francisco, CA 94143, USA.

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