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Heat Shock Proteins Functioning as Molecular Chaperones: Their Roles in Normal and Stressed Cells [and Discussion]

William J. Welch, M.-J. Gething, A. R. Clarke, P. Viitanen, P. Lund, I. G. Haas and C. Georgopoulos

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Heat shock proteins functioning as molecular chaperones: their roles in normal and stressed cells

WILLIAM J. WELCH

Department of Medicine and Physiology, University of California, San Francisco, California 94143, U.S.A.

SUMMARY

In response to either elevated temperatures or several other metabolic insults, cells from all organisms respond by increasing the expression of so-called heat shock proteins (hsp or stress proteins). In general, the stress response appears to represent a universal cellular defence mechanism. The increased expression and accumulation of the stress proteins provides the cell with an added degree of protection. Studies over the past few years have revealed a role for some of the stress proteins as being intimately involved in protein maturation. Members of the hsp 70 family, distributed throughout various intracellular compartments, interact transiently with other proteins undergoing synthesis, translocation, or higher ordered assembly. Although not yet proven, it has been suggested that members of the hsp 70 family function to slow down or retard the premature folding of proteins in the course of synthesis and translocation. Yet another family of stress proteins, the hsp 60 or GroEL proteins (chaperonins), appear to function as catalysts of protein folding. Here I discuss the role of those stress proteins functioning as molecular chaperones, both within the normal cell and in the cell subjected to metabolic stress.

1. INTRODUCTION

The process of protein folding and association has long been considered to be a spontaneous one, governed primarily by the linear sequence of amino acids present within the polypeptide chain (reviewed by Anfinsen 1973). Recent advances in our understanding of the functions of so-called heat shock or stress proteins, however, has forced us to reconsider this notion of protein maturation. First described in *Drosophila melanogaster* over 30 years ago (Ritossa 1962), the heat shock response entails the rapid and selective increased expression of a class of proteins whose structure and function are only now beginning to be understood. Despite their designation, we know that most of the heat shock proteins are in fact expressed constitutively in all cells maintained under normal growth conditions, yet exhibit higher level expression whenever the cell finds itself under conditions unfavourable for protein folding and association. Examples of conditions leading to the increased expression of the stress proteins include the exposure of cells to: elevated temperatures, various amino acid analogues, different agents which target protein sulphydryl groups, and a large number of agents and treatments which result in a reduction of normal ATP levels. As is discussed here, and in other papers within this volume, certain members of the stress protein family have been shown to function as mediators of protein maturation, leading to their more general designation as 'molecular chaperones', reviewed by Ellis & van der Vies (1991). In what follows, I will briefly discuss those stress proteins which function as molecular chaperones, and examine why their expression increases in the cell experiencing metabolic stress. (For other reviews of the heat shock response see Craig 1985; Lindquist 1986; Morimoto *et al.* 1990; Nover 1991; Welch 1991.)

2. THE HSP 70 FAMILY

Studies at both the genetic and biochemical levels have revealed there to be a family of hsp 70-related proteins which are distributed throughout various intracellular compartments (reviewed by Welch 1990). Most members of the hsp 70 family are expressed constitutively within the normal unstressed cell, yet exhibit significantly higher expression in the cell experiencing metabolic stress. These members include: (i) the cytosolic and nuclear hsp 73; (ii) grp 78 (or BiP), a component of the endoplasmic reticulum; and (iii) grp 75 present within the matrix of mitochondria and chloroplasts. In the cell under stress, yet another member of the family, hsp 72, is expressed at extremely high levels and is found within the cytosol and nucleus. All of the related hsp 70 family members appear to be comprised of two major domains. Within the first half of the molecule, the proteins all contain an extremely similar nucleotide-binding domain, the structure of which has been determined by X-ray crystallography (Flaherty *et al.* 1990). Interestingly, this domain appears very similar to several other nucleotide-binding proteins, including actin and hexokinase. The relatively high affinity of the various hsp 70 members for ATP has facilitated

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their relatively simple and rapid purification via affinity chromatography using immobilized ATP (Welch & Feramisco 1985). In contrast to this highly conserved aminoterminal ATP binding domain, the carboxyterminal half of the various hsp 70 members exhibits somewhat more sequence diversity (reviewed by Nover 1991). This carboxyterminal domain is believed to represent the region involved in substrate binding (discussed below). In the case of the cytosolic hsp 73, computer modelling of the carboxyterminal domain predicts some homology, especially at the three-dimensional level, with the peptide-binding domain of the class I histocompatibility proteins (Sadis *et al.* 1990; Rippman *et al.* 1991). This putative similarity in overall structure is strengthened by the observation that, like the class I histocompatibility proteins, members of the hsp 70 family bind to a wide variety of small peptides *in vitro* (Flynn *et al.* 1989). This property of peptide binding may reflect the role of different hsp 70 family members in interacting with the unfolded domains of polypeptides undergoing maturation in the cell.

By the use of the techniques of metabolic pulse-chase radiolabelling and immunoprecipitation, the different members of the hsp 70 family have all been shown to interact transiently with other proteins undergoing maturation. For example, the cytosolic–nuclear hsp 73 and hsp 72 proteins have been shown to interact with nascent chains of polypeptides undergoing synthesis on the ribosome (Beckmann *et al.* 1990). The current idea is that hsp 73 or hsp 72 binds to the nascent polypeptide to prevent, or at least slow down, its folding until synthesis of the polypeptide has been completed. Once synthesis is complete, and now with all of the necessary information for folding present, the newly synthesized polypeptide is released from hsp 72 or hsp 73 in a process requiring ATP hydrolysis, probably orchestrated by hsp 70 itself (along with suitable cofactors only just now being identified). In a similar scenario, the compartmentalized forms of hsp 70 (BiP, within the endoplasmic reticulum, or grp 75, within the mitochondrial matrix) have been shown to interact with newly synthesized proteins which are being transferred from the cytosol into the endoplasmic reticulum (ER) or mitochondria, respectively (Haas & Wabl 1983; Copeland *et al.* 1986; Gething *et al.* 1986; Bole *et al.* 1986; Kang *et al.* 1990; Mizzen *et al.* 1991). Again, the prevailing notion is that this interaction of BiP or grp 75 with the translocating polypeptide helps to insure that the target protein does not commence final folding until the translocation event has been completed. Once the polypeptide is entirely inside the organelle, or once stably integrated within the organelle membrane, the particular hsp 70 chaperone is released, again a process mediated by the energy of ATP hydrolysis. The exact mechanism by which the various hsp 70 family members recognize or discriminate a so-called 'unfolded' polypeptide domain and subsequently bind to the target remains unclear. As was mentioned earlier, the various hsp 70 proteins have been shown to interact with a variety of short synthetic peptides in a relatively stable fashion, albeit with different affinities depending upon the particular sequence of amino acids present with the peptide. That hsp 73 can effectively discriminate between a properly folded and an unfolded protein has been demonstrated *in vitro*. For example, when presented with either native lactalbumin or lactalbumin which has first been reduced and carboxymethylated, hsp 73 forms a stable association with only the abnormally folded lactalbumin species; moreover, upon addition of ATP the complex rapidly dissociates (Palleros *et al.* 1991).

3. THE HSP 60 FAMILY OF STRESS PROTEINS

Yet another family of stress proteins, the GroEL/ES (prokaryotic) or hsp 60/hsp 10 (eukaryotic) proteins, has also been implicated as important components in protein maturation. First characterized in *E. coli*, the GroEL polypeptide has an apparent mass of approximately 60 kDa whereas the GroES component, exhibiting some sequence homology with GroEL, has an apparent mass of 10 kDa. Initial interest in these proteins followed from genetic studies showing that bacteria harbouring mutations in either GroEL or GroES exhibited a diverse number of phenotypes. First, mutations within the GroE locus (an operon consisting of both GroEL and GroES) resulted in the bacteria being unable to support the growth of various bacteriophages. In particular, the proper assembly of the phage head and tail proteins appeared compromised in such mutants. Subsequent studies revealed that these GroE mutants displayed several other defects including impaired DNA and RNA synthesis, a block in cell division, and a reduction in overall proteolysis. Not surprisingly, both a functional GroEL and GroES appear essential for the growth of *E. coli* at all temperatures (extensively reviewed by Georgopoulos *et al.* (1990)).

Biochemical studies have shown that both GroEL and GroES exist as higher ordered, well-defined structures. The GroEL complex consists of 14 identical subunits arranged as two 7-membered rings, resembling two doughnuts stacked one on top of the other. Similarly, GroES exists as a single 7-membered ring-like structure. New insights into the function of these proteins followed from observations that a plant chloroplast protein, termed the rubisco subunit binding protein has high sequence similarity to GroEL (Hemmingsen *et al.* 1988). Rubisco (short for ribulose 1,5,bisphosphate carboxylase-oxygenase) is a large oligomeric complex, consisting of eight large subunits and eight smaller subunits, which catalyses the fixation of CO_2 within the plant chloroplast. Biochemical studies have shown that the GroEL-related rubisco subunit binding protein catalyses the orderly association of the rubisco large and small subunits into their mature and biologically active rubisco complex (Ellis 1990). Subsequent studies demonstrated that the *E. coli* GroEL and GroES proteins could effectively substitute for their plant counterparts in facilitating the assembly of active cyanobacterial rubisco within *E. coli* (Goloubinoff *et al.* 1989). The term 'chaperonins' has been suggested to describe this particular

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group of molecular chaperones (Hemmingsen *et al.* 1988).

Recent work has revealed the existence of other proteins within eukaryotic cells which appear similar to the bacterial and plant GroEL proteins. Moreover, there now is considerable evidence that these related proteins may have a more widespread role in protein maturation. Perhaps one of the better-characterized members of these eukaryotic GroEL-related family members is the 60 kDa heat shock protein present within mitochondria (McMullin & Hallberg 1987). This protein is synthesized within the cytosol and is then transferred into the matrix of the mitochondria where it assembles into the classical GroEL-like double-doughnut structure. Both biochemical and genetic studies have shown hsp 60 to be a critical component for protein maturation within the mitochondria (Cheng *et al.* 1989; Mizzen *et al.* 1991). In addition to facilitating the orderly association of monomeric proteins into their final oligomeric structures, recent experiments suggest that hsp 60 may actually participate in the earliest steps of monomeric protein folding as well. Such activities also require the participation of a second mitochondrial component, hsp 10, which appears homologous to the smaller bacterial GroES protein (Ostermann *et al.* 1989; Lubben *et al.* 1990; Mendoza *et al.* 1991).

Owing to its apparent role in facilitating protein folding and association, and because of the widespread occurrence throughout the cell of various hsp 70-related proteins, several investigators have speculated that other hsp 60-related proteins may exist within other cellular compartments. Indeed, recent work has shown that a protein in rodents, first identified by genetic means and shown to be crucial for mouse spermatogenesis, is in fact a cytosolic form of hsp 60 (Silver *et al.* 1979). This protein, referred to as TCP-1, displays the classical double-doughnut-like structure, exhibits ATPase activity, and binds to unfolded polypeptides, characteristics reminiscent of all the classically defined hsp 60 or GroEL family members (Trent *et al.* 1991; Lewis *et al.* 1992). By using an *in vitro* translation system, two groups have recently reported that this TCP-1 protein is clearly an important component for the folding and assembly of two cytosolic proteins, actin and tubulin (Gao *et al.* 1992; Yaffe *et al.* 1992). Consequently, one wonders whether additional forms of hsp 60 will be discovered, for example, a form present within the nucleus as well as one within the endoplasmic reticulum.

4. MEMBERS OF THE HSP 70 AND HSP 60 FAMILIES MAY WORK IN TANDEM TO FACILITATE PROTEIN MATURATION

Considering that members of both the hsp 70 and hsp 60 families interact transiently with newly synthesized proteins, it seems plausible that the two families of molecular chaperone may function in a sequential fashion to facilitate protein maturation. This idea originally followed from studies examining the biogenesis of various mitochondrial proteins. Specifically, following their synthesis in the cytosol, mitochondrial proteins are transferred into the matrix of the mitochondria in a relatively unfolded state. Indirect evidence has implicated a role for the cytosolic forms of hsp 70 in stabilizing the newly synthesized mitochondrial precursors in an unfolded or 'translocation-competent' state (Chirico *et al.* 1988; Deshaies *et al.* 1988). Also important is the presence of the so-called mitochondrial 'signal sequence', usually present at the extreme aminoterminus and containing a preponderance of basic amino acids. Studies by Randall and colleagues have shown that such signal sequences themselves function to retard or prevent the folding of the mitochondrial precursor protein (Park *et al.* 1988). As the mitochondrial precursor protein enters the matrix of the mitochondria, its signal sequence is removed and the translocating protein appears to form a complex with the mitochondrial form of hsp 70 (Kang *et al.* 1990; Mizzen *et al.* 1991). Similar to the proposed role for the hsp 70-related BiP protein present within the endoplasmic reticulum, this mitochondrial form of hsp 70 binds to, and thereby prevents the premature folding of, the translocating polypeptide. Once entirely inside the organelle (or in the case of transmembrane proteins, once the stop transfer sequence has become integrated into the mitochondrial membrane), and now with all of the necessary information for folding present, the newly synthesized mitochondrial protein is probably handed over to the hsp 60 complex, where folding (or association with other mitochondrial components) commences. Recent results from Ulrich Hartl's laboratory suggest that a similar sequence of events may occur in bacteria. By using an *in vitro* system, these investigators have observed that protein folding occurs through the successive actions of DnaK (bacterial hsp 70), along with its co-factors DnaJ and GrpE, followed by GroEL/ES (Langer *et al.* 1992). Consequently, a picture is emerging whereby protein folding and assembly is orchestrated by the sequential action of molecular chaperones which are probably present within all intracellular compartments.

5. WHY DOES THE CELL INCREASE ITS LEVELS OF MOLECULAR CHAPERONES AFTER EXPERIENCING METABOLIC STRESS?

As was mentioned earlier, despite their designation as heat shock or stress proteins, it is clear that most of these proteins are expressed constitutively in the cell and represent essential components for protein maturation. In fact, it is somewhat ironic that most of what we know concerning their biological function has followed from studies examining their role within the normal, unstressed cell. As was first described over 30 years ago, simply raising the temperature of cells slightly above their physiological norm results in the rapid and rather selective increased expression of the heat shock proteins (Ritossa 1962; Tissieres *et al.* 1974). Subsequent studies have revealed that a multitude of other metabolic insults, including various amino acid analogues, agents such as arsenite or β-mercaptoethanol, both of which target protein sul-

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phydryl groups, or agents and treatments that affect overall energy metabolism, all result in the cell expressing high levels of the heat shock or stress proteins (reviewed by Nover 1991). How can such a multitude of different metabolic insults lead to such similar changes in gene expression? In examining this question, Hightower (1980) noted that a common property linking these various agents and treatments was their ability to interfere with protein folding and association. Consequently, he suggested that, whenever the cell found itself under conditions where proteins began to fold abnormally (or even denature), a stress response would be initiated. Subsequent support for this idea was the observation that simply injecting denatured proteins into living cells was sufficient to activate a stress response (Ananthan *et al.* 1986).

Now knowing that at least some of the stress proteins function as molecular chaperones, we are finally beginning to understand why these proteins are up-regulated in the cell experiencing metabolic stress. Under normal growth conditions, the various members of the hsp 70 and hsp 60 families of stress protein interact with other cellular proteins undergoing maturation. Importantly, the interactions of the molecular chaperones with their target polypeptides are transient. Once the particular target protein has achieved its final folded conformation, the particular chaperone is released, and is now available for a new round of substrate interaction. Recent data from our laboratory suggest that such events involving the molecular chaperones are compromised in the cell experiencing metabolic stress (Beckmann *et al.* 1992). For example, when cells are exposed either to sodium arsenite or an amino acid analogue (two very potent inducers of the stress response), newly synthesized proteins are still observed to interact with their cytosolic hsp 70 chaperone. In contrast to the situation within the normal cell, however, under these conditions leading to stress, the newly synthesized proteins remain in relatively stable complexes with their hsp 70 chaperone.

We suspect that when a newly synthesized protein incorporates an amino acid analogue it experiences difficulties in assuming its properly folded state. As a consequence, the newly synthesized protein continues to look 'unfolded' and therefore remains bound to its chaperone. Consistent with the idea that folding is compromised in cells exposed to amino acid analogues is the fact that most of the analogue-containing proteins exhibit abnormally short half-lives (Schmike & Bradley 1975; Prouty *et al.* 1985). As more of the analogue-containing proteins are synthesized and complexed with hsp 70, the cells' level of cytosolic chaperone activities is reduced. The cell somehow senses this reduction in its available levels of hsp 70 chaperone and responds by the activation of the stress response. Through such a response, the cell redirects its priorities, leading to the rather selective expression of new chaperones (i.e. stress proteins). Unfortunately, in the case of amino acid analogues, the new chaperones produced themselves incorporate the analogue and therefore are rendered nonfunctional (Li and Laszlo 1985). As a result, even after removal of the amino acid analogue, the cell will continue to selectively synthesize what are now functional forms of the stress proteins for many hours (Welch & Suhan 1986). Apparently only after a suitable amount of new chaperones have been produced will the cell return to its normal pattern of protein synthesis (e.g. the pattern of protein synthesis occurring before the stress event).

In a similar but nevertheless distinct scenario, cells exposed to sodium arsenite also synthesize proteins which remain in a relatively stable complex with their particular hsp 70 chaperone. Under these conditions we suspect that the presence of arsenite interferes with protein maturation via its known ability to interact with vicinal sulphydryl groups. Likely targets would include the cysteine residues present within the maturing polypeptide or, alternatively, the intracellular reducing agent, glutathione (or both). Consequently, in the presence of arsenite, inappropriate cross-linking of cysteine residues may occur within the maturing polypeptide (as well as perhaps in mature proteins), thereby perturbing its proper folding and resulting in its relatively stable interaction with hsp 70. Indirect support for this idea is the fact that addition of the reducing agent β-mercaptoethanol to the arsenite-treated cells resulted in newly synthesized proteins exhibiting their normal transient interaction with hsp 70. Moreover, these cells now did not undergo a stress response (Beckmann *et al.* 1992).

Finally, in the case of heat shock, our studies revealed there to be two major populations of labile targets: both newly synthesized proteins and mature polypeptides. For example, after a 43°C, 90 min heat shock treatment, a significant amount of both classes of protein were found to partition within the insoluble fraction following lysis of the cells in non-ionic detergents. The relative amount of this detergent-insoluble material increased as a function of the severity of the heat shock treatment. Present within this detergent-insoluble fraction were significant amounts of cytosolic hsp 70 (Beckmann *et al.* 1992). Whether hsp 70 itself was rendered unfolded or binds to other proteins as they began to denature is currently under study. Whatever is the case, again the net result is a reduction in the levels of available hsp 70 chaperone activities and therefore a corresponding induction of the stress response.

6. UNRESOLVED QUESTIONS AND FUTURE DIRECTIONS

Several unresolved questions remain concerning the function and regulation of molecular chaperones within the cell experiencing stress. Firstly, in cells exposed to amino acid analogues, where newly synthesized and analogue-containing proteins remain in a relatively stable complex with their hsp 70 chaperone, one wonders exactly how the cell recognizes that these analogue-containing proteins are non-functional. Secondly, once recognized as being non-functional, how are the analogue-containing proteins subsequently targeted for degradation? Might the molecular chaperones actually participate directly in this

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process of degradation? For example, might hsp 70 be involved in the presentation of the analogue-containing proteins to the appropriate proteolytic system? If the proposed scenario, in which a member of the hsp 70 family presents a newly synthesized and unfolded protein to a member of the hsp 60 family for subsequent folding, is correct, what are the sequence of events involving an amino acid analogue-containing protein? Specifically, if presented with a newly synthesized amino acid analogue-containing protein unable to fold properly, how does hsp 60 deal with the protein? Does the cell use some type of 'quality control' system whereby proteins unable to fold properly are recognized as such, and are therefore never presented to a member of the hsp 60 family?

Yet another question concerns whether molecular chaperones participate in the refolding of proteins denatured as a consequence of the stress event. For example, we find that many mature proteins are rendered detergent insoluble in cells given a brief hyperthermic treatment. With time of recovery of the cells placed back at their normal growth temperature, the amount of protein present within the detergent-insoluble fraction decreases. Are such proteins initially denatured (therefore partitioning into the detergent-insoluble fraction) and subsequently renatured through the action of the molecular chaperones? Or are they targeted for degradation? The idea that heat shock proteins function to 'resurrect' denatured proteins back into their properly folded and biologically active conformation has been a popular one for some years (Pelham 1986). Although there are some reports that denatured proteins can be rescued both *in vivo* and *in vitro* by the action of heat shock proteins serving as molecular chaperones, considerably more data are required before reaching a firm conclusion to this interesting question (Gaitanaris *et al.* 1990; Skowra *et al.* 1990).

A final question concerns whether molecular chaperones will turn out to be important as it relates to other biological phenomena involving protein association–disassociation events. I raise this question because many proteins, even hours after their synthesis, may require the participation of molecular chaperones for the acquisition of their proper biological activity. One example concerns the mechanism by which certain transcription factors are assembled into their biologically active conformation. Recent studies have demonstrated that many transcription factors, present in an inactive form as monomers, are 'activated' via oligomerization. In many cases oligomerization is believed to occur via specific domains which are characterized by a preponderance of hydrophobic residues arranged along one side of an α-helix. These so-called 'leucine zippers' appear to function by facilitating the association of the inactive transcription monomers into their biologically active oligomeric structures (reviewed by Landschulz *et al.* 1988). Consequently, one wonders about the mechanisms involved in this pathway of transcription factor activation. Specifically, in its monomeric form, how is the leucine zipper situated within the protein? Is it, like other hydrophobic domains, buried inside the molecule? If so, upon activation, does the transcription factor monomer undergo a conformational change to allow for the exposure of the leucine zipper and subsequent oligomerization? If so, are molecular chaperones mediating such conformational changes? Alternatively, might the leucine zipper always be present on the 'surface' of the monomer but be masked by another macromolecule (like a chaperone) until activated to oligomerize? Similarly, might there be other proteins in the cell whose biological activities are regulated via association–disassociation events mediated, at least in part, by molecular chaperones? Examples include components of the cell cycle, proteins involved in DNA replication and mitosis, and finally other dynamic proteins such as components of the cytoskeleton, and those involved in adhesion. My own suspicion is that we have only begun to address the many areas in protein biochemistry and cell biology where molecular chaperones will prove to have an important impact.

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Discussion

M.-J. Gething (*Howard Hughes Medical Institute, University of Texas, Dallas, U.S.A.*). Does Professor Welch know the maximum number of different hsp 70 proteins that exist?

W. J. Welch. This is a difficult question to answer. For example *in vitro* translation of a single cDNA gives rises to six to nine isoforms and we have not managed to determine why so many are produced.

M.-J. Gething. Is there one gene for hsp 72 and one for hsp 73?

W. J. Welch. There are at least two inducible genes and there could be more.

A. R. Clarke (*Molecular Recognition Centre, University of Bristol, U.K.*). Does Professor Welch interpret his experiments to mean that constitutive hsp 73 subunits and inducible hsp 72 proteins occur in the same oligomer?

W. J. Welch. We see that many newly synthesized proteins interact with both the hsp 72 and the hsp 73 proteins. We also observe that the hsp 72 and hsp 73 proteins interact with each another to form what appear to be heterodimers. The constitutive hsp 73 also forms a homodimer or higher order structures as well.

A. R. Clarke. Are both types of heat shock 70 protein in the higher order structures?

W. J. Welch. We have not looked at that. The interesting question to address is the form of hsp 70 which interacts with newly synthesized polypeptides. For example if this form should be a dimer, do the two components dissociate from each other before release from the polypeptide on addition of ATP?

P. Viitanen (*Du Pont de Nemours, Wilmington, U.S.A.*). When Professor Welch observes that both hsp 72 and hsp 73 are immunoprecipitated by antiserum to either protein, how can he rule out that these two proteins are bound to the same polypeptide instead of to each other?

W. J. Welch. When we carry out the immunoprecipitation with pulse-labelled cells at the chase stage we see no significant number of labelled polypeptides coprecipitating with the hsp 72 and 73.

P. Lund (*School of Biological Sciences, University of Birmingham, U.K.*) Professor Welch observes discrete labelled bands of protein that are immunoprecipitated by antiserum to hsp 70 protein. Does he interpret this to mean that the hsp 70 protein is binding only to fully synthesized proteins and not to nascent chains?

W. J. Welch. We presume this is the case as we can clearly see discrete bands.

I. G. Haas. (*Institute of Genetics, University of Cologne, F.R.G.*). Returning to the evidence that hsp 72 and hsp 73 bind to each other, Professor Welch's argument based on the pulse-chase experiments does not hold true, because in the chase part of the experiment he is immunoprecipitating from a steady state situation and thus may not see ligands which bind both the hsp 72 and the hsp 73.

W. J. Welch. I agree that they may be other proteins binding both the hsp 72 and the hsp 73 at the same time.

I. G. Haas. How was it shown that the antiserum to hsp 73 does not react with hsp 72?

W. J. Welch. By immunoblotting and by using immunofluorescent methods on cells that do or do not contain one or both of these proteins.

I. G. Haas. This type of evidence does not rule out that the antiserum to one type of heat shock protein reacts with the native form of the other type of heat shock protein.

W. J. Welch. We observe no gel shifting after treating the purified native protein with the antiserum to the other type of protein.

C. Georgopoulos. (*Biomedical Institute, University of Geneva, Switzerland*). Professor Welch suggests that hsp 72 and hsp 73 form heterodimers, but is it possible that what he is observing is one type bound to an unfolded form of the other type rather than to the native form of the other type?

W. J. Welch. This is possible. However, microinjection experiments show that the import of native hsp 72 and hsp 73 proteins into the nucleus can be blocked by antibody to either protein; of course this type of experiment is not highly quantitative.

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