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Mammalian heat shock protein families. Expression and functions

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Abstract. When prokaryotic or eukaryotic cells are submitted to a transient rise in temperature or to other proteotoxic treatments, the synthesis of a set of proteins called the heat shock proteins (hsp) is induced. The structure of these proteins has been highly conserved during evolution.
The signal leading to the transcriptional activation of the corresponding genes is the accumulation of denatured and/or aggregated proteins inside the cells after stressful treatment. The expression of a subset of hsp is also induced during early embryogenesis and many differentiation processes.
Two different functions have been ascribed to hsp:
– a molecular chaperone function: chaperones mediate the folding, assembly or translocation across the intracellular membranes of other polypeptides, and
– a role in protein degradation: some of the essential components of the cytoplasmic ubiquitin-dependent degradative pathway are hsp.
These functions of hsp are essential in every living cell. They are required for repairing the damage resulting from stress.
Key words. Heat shock proteins (hsp); chaperones; protein degradation; ubiquitin.

Introduction

The first contribution of this volume has already dealt with the discovery and general description of the cellular heat shock response in *Drosophila*. Two books[46, 51] and a recent meeting review[30] give up-to-date information on heat shock proteins (hsp) and heat shock gene expression.
The following contribution will focus on the heat shock response in mammalian, mainly mouse cells. Our aim is to draw attention to the following, less well-known points:
– Heat shock protein synthesis is only one phase of a general adaptive response towards heat shock and similar stresses.
– Heat shock proteins are members of large families of proteins which have similar functions, but different expressions.
– Heat shock protein synthesis can be regulated in a specific way, independently of any stress treatment, in different physiological conditions, for instance during gametogenesis or early development.
– Heat shock proteins and related proteins fulfil essential functions in the normal cell.
– The same functions are required in stressed cells.

The mammalian heat shock response

When a mouse cell is submitted to proteotoxic treatments, such as a transient rise in temperature (transition from 37 °C to 42–43 °C for 15 min), or to chemical stresses such as addition of sodium arsenite, ethanol, heavy metals or amino acid analogs, it is possible to distinguish three successive phases in the cellular response to these aggressive treatments:
– The first phase, immediately following the beginning of the stress treatment, corresponds to an alteration phase. There is a general decrease in gene transcription and mRNA translation. There are also changes in cell morphology and modifications of chromatin and of the cytoskeleton. Some enzymatic activities are decreased or lost, probably due to the denaturation of the corresponding proteins by the stress treatment. However, other enzymes, such as some protein kinases, are activated during this first phase. The activation of these protein kinases does not require protein synthesis. These protein kinases might fulfil an adaptive function. For instance, activation of eiF2 kinase might be partly responsible for the decrease in protein synthesis observed after stress[14]. This decrease would prevent the synthesis of incorrectly folded proteins during stress treatment. Recently an-

other protein kinase activated by heat shock and other stresses has been characterized by Vincent Legagneux [39]. This kinase is able to phosphorylate a peptidic motif which is highly repeated in the C-terminal part of the largest subunit of RNA polymerase II. In fact, the phosphorylation state of RNA polymerase II increases during heat treatment [17]. Since the extent of phosphorylation of RNA polymerase changes during the different phases of gene transcription, the hyperphosphorylation of RNA polymerase might participate in the modification of transcription observed during the heat treatment.

– In the second phase, the stress treatment leads to the transcriptional activation of the so-called heat shock genes. The mRNAs coding for hsp are preferentially translated, which leads to the accumulation of hsp inside the cells.

– In the third phase, the recovery phase, gene transcription and mRNA translation return to their normal level. The cell recovers a normal morphology, and enzymatic activities which were either decreased or increased during the first phase of the heat treatment return to their initial values.

This general description shows that the synthesis of hsp is only a part of a general adaptive response toward stresses. The timing of their synthesis suggests that they play a role in the repair of cell damage resulting from the heat treatment.

Description of mammalian heat shock proteins

In mouse cells, as shown by 1-dimensional or 2-dimensional gel electrophoresis, the major hsp are [41, 46]:

– one protein of 110 kDa,
– two proteins of 90 kDa, called hsp86 and hsp84, and
– two proteins of about 70 kDa, called hsp68 and hsp70.
– one protein of 60 kDa.

To these major proteins, one must add a 27-kDa hsp, homologous to the low mol. wt *Drosophila* hsp, not detectable with ^{35}S methionine labeling. This hsp is structurally related to the α–crystallins of the lens. αB-crystallin itself is an hsp [33]. A 47-kDa hsp, located in the endoplasmic reticulum and having an affinity for collagen, has been described [48]. There is also a 8-kDa hsp involved in protein degradation, ubiquitin (see section on 'Heat shock protein functions in the normal cell' below), and other minor hsp less easily detectable by PAGE.

Among the hsp, some are induced during the heat treatment and are undetectable in the normal unstressed cells: such is the case for hsp68 and hsp110. Others are already synthesized at a constitutive high level before the heat treatment and their synthesis is only increased after the stress: such is the case with the 60-kDa hsp, the 70-kDa hsp (also called hsc70 [c for cognate] or hsp73), by different authors and in other organisms or proteins of the 90-kDa family.

Some of the hsp have been highly conserved during evolution: mouse hsp60 is homologous to the major prokaryotic hsp, GroEL (also called hsp65 in most prokaryotic organisms) whereas hsp68 and hsp70 are homologous to the DnaK protein of *E. coli*.

The mammalian hsps can be gathered into families. The two major families are the 70-kDa and 90-kDa families. The 70-kDa family contains many different genes, coding for different proteins expressed under different conditions and in different cell compartments. For instance, in mouse cells, the presently known members are:

– hsp68, the major heat inducible hsp, and
– hsp70 or hsc70 which is constitutively expressed and only slightly increased by stress.

There are also two other proteins not induced by the heat treatment:

– P75 (or mthsp70), which is found inside the mitochondrial matrix [32] (whereas the two preceding proteins are cytoplasmic and nuclear), and
– Grp78 or BiP, located in the lumen of the endoplasmic reticulum. The synthesis of this protein is induced by a decrease in glucose concentration in the cell culture medium or by the addition of Ca^{++} [47].

The number of genes coding for these different proteins is presently unknown, but probably high. For hsp68 more than 5 genes have been described, two of them coding for specific forms of hsp68 expressed only during gametogenesis [70, 71].

Regulation of heat shock protein gene expression

The transcriptional activation of the heat shock genes is correlated in eukaryotes with the presence in their promoters of one or several consensus sequences, which have been called hse for heat shock elements. These sequences are contiguous arrays of the 5-bp sequence nGAAm arranged in alternating orientations [69]. On these sequences, factors called hsf for heat shock factors bind during and after the heat treatment. These factors preexist in the cell in an inactive form before the heat treatment. The stress leads to the accumulation of denatured, abnormal proteins within the cytoplasm. The formation of these denatured proteins is the signal responsible for the activation of hsf by a yet unknown mechanism.

The deactivation of hsf is also not yet understood. Hsp probably play a major role in the switching-off of the heat shock response and in the arrest of hsp gene transcription [16, 69].

As previously seen in *Drosophila*, the expression of some members of the hsp family can be regulated independently of the overall heat shock response. In mammalian organisms, a very specific expression of hsp is observed during gametogenesis [27, 70, 71] and, as studied in our own laboratory, during early mouse embryogenesis:

1) The first proteins synthesized after fertilization by transcription of the zygotic genome are two hsp, hsp68 and hsp70 [2]. The meaning of this early expression re-

mains unknown. It is not clear whether the genes involved in this early expression are the previously described heat-inducible genes, or other genes specific for a particular developmental stage.

2) At later stages of development, the 8-cell or blastocyst stage, a very high level of synthesis of the constitutively expressed hsps, hsp86, hsp84, hsc70 and hsp60 is observed [45].

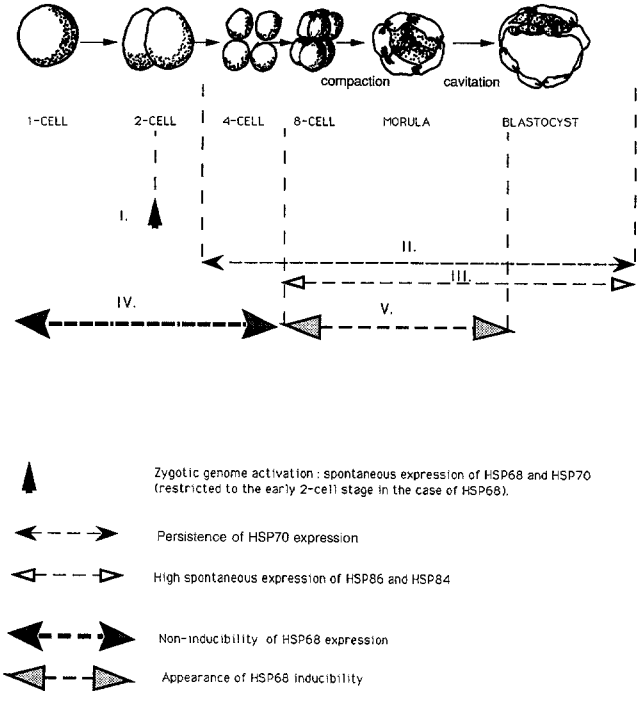
The same high level of synthesis of these constitutive hsp is seen in embryonal carcinoma EC cells, stem cells derived from tumors (teratocarcinoma), which have many biochemical properties in common with early embryonic cells. These cells allow a biochemical approach to the regulatory mechanisms underlying this very specific expression. We have shown that in EC cells at least two different mechanisms lead to the overexpression of hsc70 and hsp86:

– the increase in hsc70 level results mainly from the stabilization of its mRNA [38], and

– for hsp86 the high level of expression is due to a high level of transcription of the corresponding gene, as shown by run-on experiments [38].

Interestingly, a high level of spontaneously active hsf is found in unstressed EC cells [44] and in the mouse embryo at the blastocyst stage.

The characteristics of hsp gene expression during early mouse embryogenesis are summarized in the figure. As shown, modifications in the hsp inducibility are also observed at these early developmental stages.



Characteristics of hsp expression during mouse preimplantation development.

Heat shock protein functions in the normal cell

Two functions have been assigned to hsp:

– a chaperone function, and

– a role in protein degradation.

The best-characterized hsp at the functional level is certainly the GroEL protein from *E. coli*. This protein works in association with GroES, another polypeptide encoded in the same operon, and uses ATP as an energy source. GroEL is formed of two stacked rings of seven subunits of 60–65 kDa mol.wt. GroES is a seven subunit ring (7 × 10 kDa mol. wt). Gro EL is homologous to the ribulose bisphosphate carboxylase monooxygenase (Rubisco) binding protein, present inside the chloroplast and required for the correct assembly of nuclear and cytoplasmic subunits of Rubisco [28].

By the work of Bochkareva and colleagues [4], GroEL was shown to be loosely associated with nascent proteins. GroEL maintains these proteins in a partially unfolded conformation probably similar to the previously described molten globule structure [13, 42], an unfolded conformation with native-like secondary structures.

By using different forms of Rubisco as folding substrates, the role of the GroEL-GroES complex has been clarified [25, 26]. It does not drive the folding of proteins but it increases its efficiency by preventing aggregation of nascent, unfolded polypeptides. Different studies with β-lactamase [35] or citrate synthase [6] have confirmed this folding function of the GroEL-GroES complex. It also participates in the assembly of macromolecular structures. It facilitates the transfer of proteins across the intracellular membranes by maintaining them in a conformation competent for translocation. To designate these different functions, John Ellis and Sean Hemmingsen have chosen the word chaperone, because proteins such as GroEL-GroES perform at the biochemical level the same function as chaperones used to perform in human societies not so long ago: they "prevent improper interactions between potentially complementary surfaces and ...disrupt any improper liaisons that may occur" [19]. James E. Rothman has suggested calling these proteins PCB for Polypeptide Chain Binding proteins [62]. The word chaperonin is specifically used for the GroEL-GroES complex (also called cpn60 and cpn10).

In a eukaryotic cell, it is possible to attribute a specific chaperone function in the different cell compartments to different proteins of the hsp family. Nascent proteins interact with hsc70 inside the cytoplasm [1]. This allows a correct folding of these nascent polypeptides and facilitates the transfer of the proteins to other organelles, endoplasmic reticulum or mitochondria [11, 15]. Heat shock cognate 70 has a weak ATPase activity necessary for its release from the bound polypeptide. An autophosphorylating activity also seems to be associated with the members of the HSP70 family. The ATP-binding domain of HSC70 has been crystallized and shown to be similar

to actin[21]. The peptide binding domain shows some sequence homology with the Human Leukocyte Antigen (class I)[61]. Heat shock cognate 70 does not bind to a specific sequence[22], but probably has a higher affinity for hydrophobic aliphatic residues (as shown recently for BiP)[23]. Newly transferred proteins are chaperoned by Grp78 (BiP) inside the endoplasmic reticulum[47] or hsp60 inside the mitochondrial matrix[9,53] (in association with a recently characterized protein homologous to GroES). Another protein of the HSP family, mtHSP70, is required for the translocation of proteins inside this organelle: it binds to proteins as they are translocated, prevents their premature refolding and transfers them to hsp60 on which they will finally fold[32,65].

The second well-described function of hsp is their participation in protein degradation in prokaryotes as well as in eukaryotes. In *E. coli* one of the major proteases, La, is an hsp. In eukaryotes hsp are major components of one of the best-characterized cytoplasmic degradative pathways, the ubiquitin pathway[29,59]. Ubiquitin is activated and bound by isopeptidic bonds to the proteins which are to be degraded. Formation of multiubiquitin branched chains[8] is a signal recognized by a multicatalytic, high mol. wt protease[20,43]. Ubiquitin itself[5] and two of the E2 enzymes responsible for conjugation of ubiquitin are hsp[67]. Hsc70 might also facilitate the transfer of proteins through the lysosomal membrane and therefore participate in the lysosomal degradative pathway[10]. The ubiquitin and the lysosomal pathways are not totally distinct since ubiquitinated proteins accumulate inside the lysosomes when the lysosomal proteases are inhibited[37].

The functions of the other major hsp remain more obscure. They probably have more specific chaperone functions. Proteins of the hsp90 family bind to steroid receptors[7,66] (in conjunction with hsc70 and another hsp, hsp56) and to the protein kinases encoded by oncogenic retroviruses[52]. These interactions are necessary for the activation of the steroid receptor[56] and for the correct insertion of the oncogenic protein kinases into the plasma membrane. Heat shock protein 90 also binds to actin[34] in a calcium-calmodulin dependent way[50] and to tubulin[64] and might be implicated in the transport of proteins inside the cell.

A lot is known about the structure and modification by protein phosphorylation of the low mol. wt hsp[46]. Experiments of J. Landry have very clearly demonstrated the role of this protein in the thermoresistance of mammalian cells[36], but its biochemical function remains totally unknown.

The functions of the 110 kDa and of the newly-discovered 56 kDa HSP associated with the steroid receptores[60,63] are unknown. A homolog of the bacterial hsp DnaJ (which works in *E. coli* in association with DnaK) has recently been found in yeasts and probably also exists in higher eukaryotes[3].

Heat shock protein functions in the stressed cell

These proteins have been recruited and the genes coding for them have been placed under the control of the same regulatory sequences because these proteins are performing functions which are essential during and after heat and stress treatment. Since the effects of stress treatments are not similar in cells from different organisms, the number and nature of hsp is not identical from organism to organism: for instance, in yeasts but not in other organisms, for as yet unknown metabolic reasons, three enzymes of the glycolytic pathway have been recruited as hsp[31,40,58]. However, in all organisms so far tested, the degradative and chaperone functions of hsp appear to be essential after heat treatment:

– As suggested as early as 1984 by Hugh Pelham in his study of the modifications of nucleoli and preribosomes after heat shock[54,55], the 70-kDa chaperone hsp are able to disaggregate and renature proteins which have been inactivated and denatured during the heat treatment.

– Enzymes of the ubiquitin pathway appear to specialize in the recognition and degradation of misfolded, abnormal proteins[12].

Recent experiments in *E. coli* have confirmed in vivo and in vitro the disaggregating and refolding capacities of the chaperone hsp[24,68]. Experiments suggesting the same function for hsp in higher eukaryotic cells are less direct. Experiments performed with mouse transfected fibroblasts, expressing in a stable way reporter enzymes β-galactosidase and luciferase, have shown that the simple vision of proteins directly unfolded by heat and becoming targets for the ubiquitin pathway is naïve and does not correctly reflect what happens inside a stressed cell[18,49,57]. The major event taking place during the stress is an insolubilzation of the partially denatured proteins. This insolubilization is not always associated with the enzymatic inactivation. The insolubilized proteins are not degraded. They can resolubilize after the end of the stress treatment even in the absence of protein synthesis. Constitutively expressed hsp (in particular hsc70) are co-insolubilized with the denatured proteins. These experiments suggest that, inside the aggregates of denatured proteins, hsp play a major role in protein resolubilization, but the experimental proof of their involvement and the exact nature of the hsp involved in this process remains to be discovered by in vivo and in vitro experiments. It remains also to be determined at what time-point the cell 'takes the decision' to renature or to degrade an insolubilized protein.

Conclusion

Heat shock proteins have recently been the subject of interest of many different groups coming from different disciplines:

– On the one side, evidence for the role of hsp in polypeptide folding has stimulated a lot of physicochem-

ical studies. Many of these are already in progress, so that our fundamental knowledge about protein conformation and protein folding will probably increase very rapidly.

– On the other side, hsp appear to be involved in many different physiological processes, embryogenesis, pathogenesis and even ageing. Progress in the understanding of their roles in these complex phenomena is the area where the frontiers of our knowledge will be pushed forward in the future.

Note added in proof:

Since this paper was written, a review by Gething and Sambrook[a] and two papers have brought new information on the chaperone function of hsp:

– The existence of a new cytoplasmic chaperonin has been suggested in eukaryotes (Trent et al.[b]).

– It has been shown that DnaK, DnaJ and GroEL act successively in the protein folding pathway (Langer et al.)[c].

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