Possible nuclear functions of the major molecular chaperones of the eukaryotic cytoplasm, Hsp90

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Recent studies suggest that the chaperone function of the 90 kDa heat-shock protein, Hsp90, is restricted to help folding of nuclear hormone receptors, numerous protein kinases and damaged cytosolic proteins after proteotoxic stress. Hsp90, the most abundant chaperone of eukaryotic cytosol, may also participate in formation of cytoarchitecture and direction of cytoplasmic traffic of macromolecules. However, a small, but significant portion of Hsp90 translocates to the nucleus accompanying steroid receptors and after cellular stress. Here we summarize our present knowledge on the nuclear functions of Hsp90, and raise some suggestions for its putative role in the organization of the interphase nucleus and mitotic chromosomes.

Protein folding and molecular chaperones

FOLDING of proteins larger than 10–20 kDa often results in a conformationally trapped intermedier after the initial hydrophobic collapse of the extended unfolded structure[1,2]. These intermediers can be usually characterized by hydrophobic amino acid side chain residues exposed to their surface which renders them very sensitive to aggregation[3]. Flexibility and conformational mobility of these partially folded proteins are rather restricted if compared to the unfolded state. The comprised structure makes the further rearrangement of the inner hydrophobic core of the protein rather slow, and difficult. In case of alkaline phosphatase, the formation of the crystalline-like inner structure may last for weeks as judged by phosphorescence analysis of an inner tryptophane residue which is much slower than the almost complete renaturation of the enzyme activity occurring in the first 60 min of refolding[4]. Thus most of larger proteins need help to accelerate the organization of their inner core, to rescue them from folding traps and to protect them against aggregation. This help is provided by molecular chaperones[5].

Most of the molecular chaperones are proteins. However, there is an increasing number of reports describing the chaperone function of ribosomal and other RNAs[6–9]. Similarly, recent reports list several conformationally restricted RNA species as targets for molecular chaperones besides their original, proteinaceous target molecules[10–12]. Chaperones are ubiquitous proteins with a highly conserved structure. Their ancestors probably played an important role in enzyme evolution, enabling the development of larger proteins with enough flexibility for an 'induced fit' and for allosteric regulation[13]. In the present organisms there are many classes of molecular chaperones being able to help protein folding, to prevent protein aggregation and to solubilize 'looser', reversible protein aggregates[14].

Help of protein folding is also necessary after a great variety of environmental stresses. The expression of most molecular chaperones is induced after cellular stress to provide a more efficient protection and recovery. Heat shock is considered to be the 'archetype' of proteotoxic stress – therefore most molecular chaperones are called heat-shock proteins (Hsps).

Role of Hsp90: The present paradigm and its limitations

The 90 kDa heat-shock protein, Hsp90, is known to participate in the *in vivo* folding of most *nascent* nuclear hormone receptors and many protein kinases[15]. However, it is the most abundant chaperone of the eukaryotic cytoplasm comprising 1–2% of cytoplasmic proteins and its presence is mandatory for the survival of eukaryotic cells. These features of Hsp90 suggest a more general role for this protein. Hsp90 behaves as an organizer of the 'foldosome', a highly dynamic chaperone complex of the eukaryotic cytosol, and displays a chaperone activity in a great variety of *in vitro* systems[15,16]. On the basis of these features, Hsp90 was suggested to be a general chaperone of the eukaryotic cytosol[17]. This assumption would explain why Hsp90 is required for the survival of eukaryotic cells and why it is expressed constitutively in such a large amount. However, the recent discovery that the folding of nascent proteins occurs mostly co-translationally in eukaryotes, whereas in eubacteria it is mainly a post-translational event[18], shows that in eukaryotes, where Hsp90 has vital functions, the need for general post-translational chap-

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eroning is limited. In contrast, in eubacteria, where the need for chaperones is much more expressed, Hsp90 deletion is not lethal[19]. Moreover, Hsp90 (in contrast to Hsp70) has been observed as part of the ribosome-attached chaperone machinery only in case of steroid receptors and some protein kinases[15,16]. As it is shown in Figure 1, most eukaryotic proteins probably require only the help of the ribosome for their *de novo* folding. In contrast, folding of most components of the cytoskeleton is aided by the cytoplasmic Hsp60 machinery, while some nuclear hormone receptors and protein kinases develop their unstable structure utilizing the help of the Hsp90-associated chaperone machinery, the foldosome (Figure 1). Thus the proposed 'general chaperone' role for Hsp90 probably does not provide a sufficient explanation for the high abundance and crucial importance of this protein in eukaryotes.

The organizational role of Hsp90 in the foldosome, in signalling events, and in proteolytic degradation, together with its participation in various forms of the cytoskeletal structure raise the possibility that Hsp90 may participate in the maintenance and remodelling of the cytoarchitecture by guiding of some selected *de novo* synthesized or damaged targets to their proper destination within the cytoplasm[15,16]. Hsp90 may be similar in this respect to the other major cytoplasmic chaperone, the Hsp60-TCP1 protein[20]. Another possible role of Hsp90 to explain its crucial requirement for the survival of eukaryotic cells may lie in its role in nuclear, or nucleus-related functions.

Hsp90 in the cell nucleus

About 5 to 10% of cellular Hsp90 is known to be localized to the cell nucleus. An additional fraction of Hsp90 translocates to the nucleus after a single or repeated heat shock[16,21]. At first sight a few per cent of a protein may seem negligible; however, Hsp90 is one of the most abundant proteins in almost all cells, so that even a small proportion may be significant. Hsp90 harbours a bipartite nuclear localization sequence preceded by a poly-glutamic acid tract shown to facilitate the nuclear translocation of nucleoplasmin[22,23]. However, these signals are buried, and can be activated only after an exposure exemplified by the construction of deletion mutants[24]. Furthermore, Hsp90 also has numerous sequences similar to other known 'traditional' or 'alternative' nuclear import and export signals[16]. This, together with other initial findings suggests that, like the steroid receptors and Hsp70, Hsp90 is also constantly shuttling back and forth between the cell nucleus and the cytoplasm and participates in the nuclear import and export mechanism.

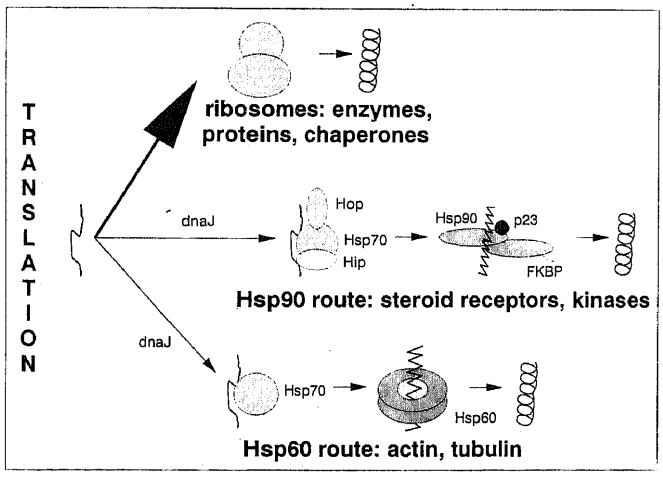

Figure 1. Folding routes of nascent proteins in the eukaryotic cytoplasm. Abbreviations: dnaJ, Hip: co-chaperones of the 70 kDa heat shock protein, Hsp70; Hop: connecting-protein and co-chaperone of Hsp70 and Hsp90; p23: 23 kDa Hsp90-associated chaperone to prevent the premature dissociation of the target protein from Hsp90-chaperone complex, the foldosome; FKBP: Hsp90-associated immunophilin.

Hsp90 as a DNA/RNA-binding protein

Hsp90 is able to bind both DNA and RNA with relatively low affinity[16]. Interestingly, a 60 amino acid stretch around the LKVIRK epitope of Hsp90 displays significant homology with the ssDNA/RNA-binding region of several plant viruses[24] and the unstability-determinant 3′ UTR RNA-binding segments of *Drosophila* and *C. elegans* proteins[25]. Hsp90 associates with specific heat-shock puffs (hsr-omega) in Drosophila polytene chromosomes[26,27]. Binding of Hsp90 to 3′ UTR regions of various RNAs may have several interesting consequences: Hsp90 may participate in the protection of mRNAs after heat shock, a role proposed for the Hsp90-containing heat shock granules[28], may be involved in the localization/transport of mRNAs during embryonic development and normal cell growth[29–31], and may also regulate mRNA degradation by the eukaryotic homologues of RNAase E and K (which are associated with the Hsp60-homologue, GroEL) or by the proteasome-associated RNAase activity[32–33]. Recent findings indicate that the endoplasmic reticulum is also involved in mRNA localization[31]. This raises a possibility for the involvement of Grp94, a protein highly homologous with Hsp90 in the endoplasmic reticulum, in the localization of mRNAs. These putative functions are supported by the fact that both proteins bind to actin filaments[34].

Hsp90 as a modulator of transcription

Segments of the highly charged region of Hsp90 following its *N*-terminal domain strongly resemble those of the DNA structure[35]. Thus it is not surprising that Hsp90 interacts with histones and several transcription factors. If Hsp90 forms a low-affinity, transient complex

with the respective transcription factor, it usually enhances DNA-binding. Thus Hsp90 promotes DNA-binding of several helix–loop–helix transcription factors, such as MyoD1 or E12 (refs 36, 37). By contrast, if Hsp90 forms a stable complex with the transcription factor, it decreases or prevents DNA-binding. Thus a stable complex with Hsp90 in the absence of the respective hormone prevents DNA-binding of most nuclear hormone receptors[15].

Interaction of Hsp90 with histones

Hsp90 avidly binds histone molecules and induces a tighter, salt-resistant structure of rat liver chromatin[38,39]. Hsp90 possesses a poly-glutamic acid sequence similar to that of nucleoplasmin, which plays an important role in the assembly of nucleosomal structure[16,22]. In agreement with this homologous sequence, circular dichroism measurements of DNA and added histones indicated that Hsp90 may have a nucleoplasmin-like activity by promoting the assembly of histones and DNA at physiological salt concentrations[38].

The 'tetratricopeptide connection'

Hsp90 binds various proteins containing a tetratricopeptide repeat (TPR) domain, such as the Hsp90–Hsp70 connecting protein, Hop (p60), and the Hsp90-binding immunophilins (FKBP52, Cyp-40, PP-5). One of these TPR-proteins, FKBP52, has been suggested to participate in directing steroid receptor holo-complexes to the cell nucleus. Binding of the TPR-containing 'directing' components is mutually exclusive, meaning that Hsp90 can form a complex with only one of them[15]. These and other recent findings raise the possibility that the above proteins play a decisive role in directing Hsp90 and its specific targets along cytoplasmic trajectories. However, almost all the initially identified members of the TPR-containing protein family participate in mitosis (as members of the anaphase promoting complex), transcription (as members of the transcription repression complex), splicing and nuclear protein import[40,41]. Though a direct interaction of these 'original' TPR-proteins with Hsp90 has not yet been demonstrated, they are likely to participate in the various nuclear functions of Hsp90.

Interestingly, the enzyme responsible for *O*-glycosylation, the *O*-linked *N*-acetyl glucosamine-transferase, is also a tetratricopeptide repeat containing protein. *O*-glycosylation is an important regulatory modification, which occurs mainly in the cell nucleus and in many cases has a reciprocal relationship with phosphorylation[42]. Grp94, a protein highly homologous with Hsp90 in the endoplasmic reticulum, has been reported to contain *O*-linked oligosaccharides[16]. These findings may reflect that a TPR-mediated *O*-glycosylation is an important regulatory element of both Grp94 and Hsp90 as well as some of their associated proteins, like casein kinase II[43].

Hsp90 during mitosis

Some of the DNA-, histone- and other nuclear protein-related effects of Hsp90 may be mediated by the relatively minor amount Hsp90 present or translocated to the cell nucleus. However, a much better chance for Hsp90-histone or -DNA interactions occurs in the mitotic process, where the nuclear barrier for the bulk of Hsp90 is abrogated. In agreement with this, Hsp90-α of *Saccharomyces cerevisiae* has been identified as an early meiotic gene induced by the IME1-IME2 transcriptional cascade[44]. Binding of Hsp90 to ssDNA may reflect its role in the preservation of 'gene expression cellular memory' during mitosis[45]. Its *in vitro* role in the stabilization of the chromatin structure[38] may reflect its participation in the chromosome condensation/decondensation events. An active role of Hsp90 in mitosis would explain the requirement of the large amounts of this protein in eukaryotic cells.

Conclusions

We have summarized the putative roles of Hsp90 in the cell nucleus in Figure 2. Involvement in protein and RNA export and import, participation in mRNA localization and degradation, modulation of transcriptional events during interphase and mitosis as well as mediation of chromatin condensation and decondensation are all hypothetical roles for this cytoplasmic chaperone. All these putative nuclear or nucleus-related functions of

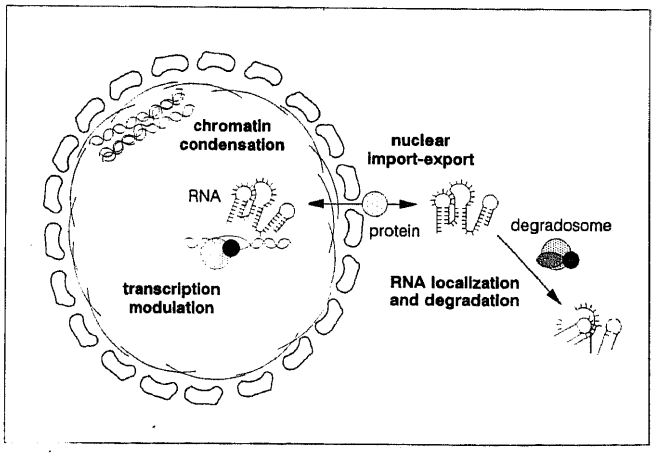

Figure 2. Putative nuclear functions of Hsp90.

Hsp90 are substantiated by several initial findings and provide numerous exciting areas for further research.

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