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Minireview

Protein-mediated protein maturation in eukaryotes

Ingrid G. Haas*

Institut für Biochemie I, Im Neuenheimer Feld 328, D-69120 Heidelberg, Germany

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Abstract Eukaryotic cells have developed particular strategies to support the critical steps in protein maturation that starts in the cytosol with the birth of a nascent polypeptide chain, and ends when the protein has reached the appropriate compartment and/or has attained its mature structure. Many of the cellular proteins that have evolved to promote maturation processes are constitutively expressed members of the highly conserved heat shock protein (hsp) family, also known as 'molecular chaperones'. Protein-mediated processes that occur in the cytosol are discussed.

Key words: Heat shock protein; Intracellular compartment; Intracellular protein targeting; Folding, in vivo; Molecular chaperone

1. Introduction

Distinct intracellular compartments within eukaryotic cells are specialized to carry out diverse cellular functions. All of them possess a distinct set of proteins that are required either to build up and maintain the organellar structure or to catalyze compartment-specific processes. The establishment and maintenance of such a sophisticated interior design require an efficient protein sorting machinery to ensure that specific functions are exerted solely at the assigned locations. Closely related to this problem is the question of where and how individual proteins are folded to gain their functional shape and integrity.

From a physical point of view, protein folding is a self-sufficient process because the amino acid sequence carries all the information required for a protein, in an aqueous solution, to fold into its native structure. In vivo protein folding, however, is a biological problem, which starts with a growing nascent polypeptide chain continuously increasing the number of amino acids available for folding. Moreover, these polypeptides are synthesized into an environment that already contains large amounts of thousands of other proteins. How, under these conditions, can a protein fold, and how can it rapidly find the appropriate organelle or the eventual partner with which it must assemble?

In fact, such processes are assisted by other proteins. The discovery that nucleoplasmin supports histone assembly was one of the first contributions that led to the concept of molecular chaperones [1,2]. Many of the molecular chaperones are ubiquitous proteins, and belong to the highly conserved heat shock protein (hsp) family (for recent reviews, see [3,4]. Although hsps were originally discovered as stress-inducible proteins [5], many family members are expressed in a constitutive fashion. Indeed, some represent major components of intracellular compartments (see below). The best studied hsps that are classified according to their molecular masses belong to the three major classes, hsp90, hsp70, and hsp60.

Here, I will briefly discuss the subcellular distribution and major tasks of the different hsps, hereby focussing on the constitutively expressed hsps only, because these are the ones that mediate normal maturation processes of proteins expressed in non-stressed cells. Details on structural and functional aspects of the various hsps including ATPase activities or the ATP dependency of in vitro chaperone function have been extensively discussed elsewhere [3,6–14]. The second part will focus on the various interactions with other proteins (listed in Table 1) that newly synthesized polypeptides transiently establish during their stay in the cytosol (Fig. 1).

2. Hsps as components of eukaryotic compartments

Hsp90 proteins (reviewed in [3]) seem to be confined to the cytosol as well as to the lumen of the endoplasmic reticulum (ER) of vertebrate cells. Cytosolic hsp90s appear to be involved in processes that regulate the functional state of cytosolic receptors or enzymes (see below). The mammalian ER-hsp 90 is known as glucose-regulated protein, GRP94, because its expression is increased upon glucose starvation of cells (for a review on GRPs, see [15]). A yeast-ER hsp90 homolog has not been described as yet and, up to now, no distinct function has been assigned to GRP94.

In contrast, hsp70 proteins were found in many intracellular compartments. Members of this protein family occur in chloroplasts, endoplasmic reticulum, mitochondria, and cytosol (see [8] for a general overview, [11] for the vertebrate, and [3] for the yeast hsp70 genes). In yeast, genes of two hsp70 subfamilies, SSA and SSB, encode the cytosolic representatives (see Table 1). One major function of constitutive hsp70 proteins is the stabilization of bound immature proteins. This serves, on the one hand, to prevent proteins from misfolding and aggregation, and, on the other, to facilitate receptor-mediated translocation of unfolded polypeptides through membranes of mitochondria, chloroplasts, lysosomes, the ER, and possibly also of peroxisomes (see below). From studies performed on bacterial chaperones, it is known that the only bacterial hsp70, DnaK, functions in concert with two other proteins known as DnaJ and GrpE, e.g. in the repair of heat-induced protein damage [16]. In eukaryotes, various homologs of DnaJ that occur in different compartments have been described (reviewed in [17]. Very recently, mitochondrial GrpE homologs have been identified as well (see [18] for references).

The structurally and functionally related group of hsp60 pro-

*Corresponding author. Fax: (49) (6221) 56-4366.

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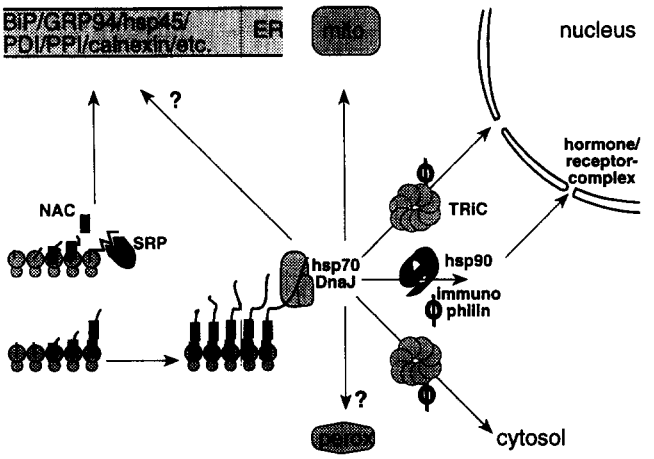

Fig. 1. Interactions of nascent polypeptides with cytosolic chaperones. The maturation steps of nascent polypeptides in the cytosol starts by NAC binding to ribosome-bound polypeptides. In case a signal is exposed, NAC binding will be replaced by SRP. Still being in the process of translation, the nascent chains will interact with hsp70 and the cytosolic DnaJ homolog. It is not clear whether the cytosolic chaperonin TRiC, which has been shown to bind with elongating luciferase, binds to only a selected pool of the newly synthesized proteins, or whether, for example, mitochondrial precursor proteins do not but remain associated with hsp70 instead. Some of the ER chaperones are indicated.

teins includes bacterial groEL (the crystal structure of which was recently solved by Braig et al. [19]), mitochondrial chaperonin 60 (cpn60), and the ribulose biphosphate carboxylase subunit-binding protein of the chloroplast stroma (Rubisco). This group is known as the chaperonins (reviewed in [7]). All of them are found as homo-oligomers of two-stacked rings, each ring with a seven-fold symmetry. The bacterial GroEL is assisted by a smaller component, GroES, which is a single ring with seven-fold symmetry. The eukaryotic homolog of GroES is the mitochondrial cpn10 [20,21]. A genetic approach in yeast demonstrated that cpn10 has an essential function [22]. Computer-based sequence comparisons were performed to search for a eukaryotic cytosolic chaperonin equivalent, which revealed that a mammalian cytosolic protein, t-complex protein-1 (TCP-1), was similar to known hsp60 proteins (approx. 60% homology [23,24]). In fact, TCP-1 and seven related subunits form a heterooligomeric ring-like structure resembling that of GroE [25–28]. The complex, termed TRiC, TCP-1 containing complex, or cytosolic chaperonin, is indeed functionally related to the chaperonins in that it interacts with immature α and β subunits of tubulin allowing the formation of $\alpha\beta$ heterodimers [29], and mediates refolding of denatured polypetides [25,26]. A TCP-1-related molecular complex isolated from oat seedlings stimulated the refolding of denatured phytochrome to a photoactive form [30]. A structural and functional comparison of the various chaperonins has been given by Kim et al. [31].

Besides these well-known groups of molecular chaperones, there are many more proteins, occasionally also modifying enzymes or isomerases, that mediate the maturation process of newly synthesized polypeptide chains. Most proteins begin their career as a growing polypeptide chain in the cytosol, from where the molecule is targeted to the appropriate location. Which set of assisting molecules comes into play depends on the final destination and function of the respective protein.

3. The cytosol as a maturation compartment

3.1. The first decisions

Most of the decisions concerning the fate of a newly synthesized protein are taken in the cytosol, suggesting that only the very first steps will be common to all nascent chains. A nascent chain associated complex, NAC, binds to presumably all polypeptide chains as they emerge from the ribosome. In case a complete ER-signal peptide is exposed by the growing chain, NAC seems to be replaced by the signal recognition particle, SRP [32]. Subsequently, and possibly restricted to polypeptides synthesized by free polysomes (and not at the membrane of the ER), the nascent polypeptide interacts with cytosolic hsp70, in vertebrates named hsc70 or hsp73 [33]. In a yeast system, it was shown that the cotranslational binding of bacterial DnaJ to two different nascent chains resulted in a translational arrest as long as the functional partners of DnaJ in *Escherichia coli*, GrpE and the hsp70 protein DnaK, were missing [4]. This is in line with translation being affected in yeast mutants that lack both cytosolic hsp70 proteins of the SSB subfamily, which are usually associated with translating ribosomes [34].

3.2. Proteins that fold in the cytosol

In addition to hsp70, both the cytosolic DnaJ homolog hsp40 and the cytosolic chaperonin TRiC were shown to be associated with nascent chains translated in a reticulocyte lysate [35]. Based on two lines of evidence, the authors propose a model for a sequential action of hsp70 and TRiC: (i) a translationally arrested short peptide (8.4 kDa) of firefly luciferase was co-isolated with hsp70 from a complex of approx. 200 kDa in size whereas a longer peptide (61 kDa) of the same protein co-precipitated with hsp70 from a 1200 kDa complex that additionally contained the chaperonin; (ii) depletion of hsp70 or hsp40 of the reticulocyte lysate affected chaperonin association with the nascent chain whereas depletion of the chaperonin did not affect hsp70 binding to the newly synthesized polypeptide. Because they found a proteinase K-resistant 23 kDa fragment derived from nascent polypeptides still associated with ribosomes, the authors propose that the folding of firefly luciferase starts during translation. However, it remains to be shown that

Table 1
Cytosolic proteins[a] involved in protein maturation

Bacteria[b]	Vertebrate	Yeast
hsp90 (HtpG)	hsp90	hsp90
hsp70 (DnaK)	hsc70 (hsp73)	Ssap1, Ssap2 Ssbp1, Ssbp2 (genes: SSA, SSB)
DnaJ	hsp40	Ydj1p
GrpE	none identified	none identified
hsp60 (GroEL)	TRiC (TCP-1-complex, cytosolic chaperonin)	TCP-1-subunit identified
GroES	none identified	none identified
None identified	none identified	NAC
Immunophilins	immunophilins	immunophilins

[a]See text for references.
[b]Bacterial equivalents are included to facilitate classification of the eukaryotic proteins.

not all polypeptides associated with translating ribosomes exhibit a 23 kDa fragment when subjected to the same experimental protocol. The involvement of a different type of proteins in the folding of nascent polypeptides was recently reported by a group that also used the reticulocyte lysate as a folding environment for in vitro translated polypeptides [36]. These studies revealed that the rapid folding of newly synthesized bacterial luciferase requires the concomitant action of two different immunophilins. These proteins usually exhibit peptidylprolyl *cis/trans*-isomerase activity (for a review on immunophilins, see [37]).

3.3. *Proteins that go to the nucleus*

Proteins can enter the nucleus as native structures, provided that they are small enough to traverse the nuclear pore by diffusion, or that they contain a nuclear localization signal, NLS (for review, see [38]. It has, however, been suggested that the cytosolic hsp70 protein has a function in mediating active import of NLS-bearing proteins into the nucleus [39–41].

3.4. *Proteins targeted to the endoplasmic reticulum*

The precursors of secretory proteins are not transported across the ER membrane in a native state. There are (at least) two alternatively acting mechanisms involved in preserving a so-called translocation-competent conformation. One is the classical pathway, in which SRP binds to the signal sequence of ribosome-bound nascent chains that results in arresting or delaying elongation. This ban is lifted by SRP dissociation fom the signal peptide that occurs at the ER membrane, which allows co-translational traslocation of nascent chains. The second, SRP-independent mechanism involves the action of cytosolic hsp70 that stabilizes the polypeptide chain in a translocation-competent form (summarized in [42]). As shown by the use of in vitro translocation systems, different chaperones differ in their ability to stimulate translocation across the ER membrane [43]. Whether or not such polypeptides fold at all prior to the translocation event remains to be elucidated.

3.5. *Proteins transported into mitochondria*

Nuclear-encoded mitochondrial proteins may also inefficiently fold as long as they reside in the cytosol. This is supported by the finding that both the precursor and the mature form of rat mitochondrial aspartate fold slowly when synthesized in a reticulocyte lysate [44]. By contrast, folding is rapid once the translation product is sequestered within the mitochondria [45]. Studies performed in *Saccharomyces cerevisiae* revealed that mitochondrial precursors require the action of hsp70 proteins encoded by the SSA genes, as well as the cytosolic DnaJ homolog, Ydj1p, to passage the mitochondrial membrane [34]. As pointed out by Craig and co-workers, it would be interesting to determine whether mitochondrial precursors that were translated with the aid of SSB-encoded proteins are subsequently transferred to SSA-proteins that support translocation across mitochondrial membranes [3].

3.6. *Proteins that build up peroxisomes*

The requirements for protein entry into peroxisomes (see [46] for a review on peroxisomes) are poorly understood. Two well-characterized peroxisome targeting signals reflect the existence of different protein import mechanisms. For example, the yeast PAS 7 gene product is involved in the translocation of peroxisomal proteins such as thiolase that possess an amino-terminal import sequence [47]. In contrast, PAS 8 mutants prevent catalase or other carboxy-terminal signal-bearing proteins from being imported [48]. Whether or not peroxisomal proteins are translocated in an unfolded conformation is not clear as yet. Interestingly, peroxisomal import could be blocked by antibodies to hsp70 as shown by experiments performed with permeabilized cells [49]. This might indicate that proteins need to be unfolded to be imported. Alternatively, the hsp70 protein might assume a function similar to that which it has in the nuclear import of folded structures [39–41], which might be to drive the assembly between the organellar protein and a specific receptor.

3.7. *Proteins that are functionally regulated*

Steroid hormone receptors, for example, are cytosolic proteins that only enter the nucleus when bound to the hormone. It has been established that the hormone-binding domain of the receptors must be bound to hsp90 for it to have steroid-binding conformation (see [50] for review). Hsp90 mutants could be isolated by screening yeast cells that have defects in glucocorticoid receptor function [51]. Direct in vivo evidence for hormone receptor/hsp90 interaction came from the results of transfection experiments. These showed that a modified hormone receptor devoid of the nuclear localisation signal (NLS) localized to the nucleus when co-expressed with a modified hsp90 that contained a NLS [52]. In fact, it is a chaperone complex including hsp70, hsp90, and an immunophilin, hsp56, that is associated with cytosolic steroid hormone receptors [53–55]. Interestingly, the yeast homologs of hsp90, hsp70 (from the SSA subfamily), and a 45 kDa immunophilin are associated with heterologously expressed mammlian glucocorticoid receptor, suggesting that hsp90 functions as part of a highly conserved macromolecular complex in eukaryotes [56]. These chaperones may exist as a preformed complex in the cytosol [57].

4. Concluding remarks

In the past few years, studies performed in yeast and vertebrate cells have predominantly been successful with respect to the uncovering of many components that assist in intracellular protein folding. However, care should be taken in establishing in vivo protein folding rules from the study of a single polypeptide chain, since different mechanisms may apply for different proteins. With respect to the problems of protein sorting, cytosolic factors might exist that, in addition to the conserved hsps, identify the proteins to be targeted to the different compartments. On the other hand, NAC and SRP may provide an example of how two 'non-specific' proteins can complement each other to increase specificity. In fact, Wiedmann and coworkers showed that, in the absence of NAC, SRP functionally binds to polypeptides that lack a signal sequence, and that it is released from the nascent chain when the complex binds to ER membranes. Therefore, it seems that it is the NAC protein *not* binding to signal peptides that confers specificity to SRP.

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