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Principles of chaperone-mediated protein folding

F. ULRICH HARTL

Howard Hughes Medical Institute and Cellular Biochemistry and Biophysics Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021, U.S.A.

SUMMARY

The recent discovery of molecular chaperones and their functions has changed dramatically our view of the processes underlying the folding of proteins *in vivo*. Rather than folding spontaneously, most newly synthesized polypeptide chains seem to acquire their native conformations in a reaction mediated by chaperone proteins. Different classes of molecular chaperones, such as the members of the Hsp70 and Hsp60 families of heat-shock proteins, cooperate in a coordinated pathway of cellular protein folding.

1. INTRODUCTION

In vitro, many unfolded proteins are able to fold to their native conformations spontaneously. This observation, first made by Anfinsen about three decades ago, demonstrated that all the information necessary to specify the three-dimensional structure of a protein is contained in its linear amino acid sequence (Anfinsen 1973). Consequently, it had been assumed that the *de novo* folding of proteins upon synthesis on ribosomes also generally occurs spontaneously. This view has changed profoundly over the past six years, due to the discovery of a large number of proteins, known as 'molecular chaperones', which are essential for cellular protein folding and occur ubiquitously in all types of cell in the cytosol as well as in various subcellular membrane compartments (Ellis 1987; Hartl *et al.* 1994).

The molecular chaperone concept

The term 'molecular chaperone' was coined for nucleoplasmin, a protein that binds to histones and mediates nucleosome assembly (Laskey *et al.* 1978). Molecular chaperone proteins of several structurally unrelated classes, many of them stress or heat-shock proteins, are now known to participate in a variety of cell functions. They facilitate *de novo* protein folding under normal growth conditions, prevent protein aggregation under stress conditions and stabilize polypeptide chains in an unfolded state for translocation across organellar membranes (Hendrick & Hartl 1993; Ellis 1994; Hartl *et al.* 1994; Stuart *et al.* 1994). Several lines of cell biological research contributed to the formulation of the novel concept of assisted protein folding: the isolation of not-yet-assembled subunits of ribulose bisphosphate carboxylase oxygenase (Rubisco) in chloroplasts as a complex with a high molecular mass binding-protein, the Rubisco subunit binding protein (RSBP), suggested a critical role of this component in Rubisco assembly (Barraclough & Ellis 1980). RSBP was later found to be the chloroplast homolgue of *E. coli* GroEL and mitochondrial Hsp60, which have been classified as members of the Hsp60 or 'chaperonin' family of molecular chaperones (Hemmingsen *et al.* 1988; McMullin & Hallberg 1988). Mutations in the genes encoding GroEL and its co-factor GroES had been reported to affect the assembly of bacteriophage particles (Georgopoulos *et al.* 1973). The oligomeric assembly of proteins imported into mitochondria from the cytosol was found to be defective in a yeast strain containing a mutated version of mitochondrial Hsp60 (Cheng *et al.* 1988). The primary function of Hsp60 was subsequently shown to be to mediate the folding of monomeric polypeptide chains (Ostermann *et al.* 1989) and the subunits of oligomeric proteins in an ATP-dependent reaction (Zheng *et al.* 1993).

As an independent line of evidence, the Hsp70s, another major class of molecular chaperones, were proposed to protect certain proteins from denaturation under heat-stress (Pelham 1986) and were shown to associate with ribosome-bound polypeptides (Chirico *et al.* 1988; Deshaies *et al.* 1988; Beckmann *et al.* 1990; Frydman *et al.* 1994). A common theme in all these studies was that the binding proteins stabilized the otherwise unstable conformations of non-native proteins which are prone to aggregation. It is now generally believed that molecular chaperones shield the hydrophobic sequences or surfaces exposed by conformational intermediates on the protein folding pathway. They do not recognize a consensus sequence motif and therefore have the ability to prevent the incorrect intra- and intermolecular folding and association of many different proteins. The Hsp70s and Hsp60s then promote correct folding by repeatedly binding and releasing their substrate proteins regulated by ATP binding and hydrolysis. In this process the molecular chaperones do not typically function as catalysts of protein folding. Generally, they increase the yield of a folding reaction rather than its speed. Once a protein has reached its native state, it no longer presents hydrophobic surfaces for chaperone binding. However, exposure to certain forms of cellular stress,

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such as heat-stress, may cause the partial or complete unfolding of proteins, leading to their renewed interaction with chaperones.

2. CELLULAR FOLDING PATHWAYS

The function of the Hsp70s and Hsp60s in protein folding is understood in considerable detail. Both groups of chaperones play an essential role in the folding of newly synthesized polypeptides in the cytosol, as well as in mitochondria and chloroplasts (Hendrick & Hartl 1993; Hartl *et al.* 1994). Here they cooperate in what appears to be a general pathway of cellular protein folding (figure 1) (Langer *et al.* 1992; Frydman *et al.* 1994; Stuart *et al.* 1994). The Hsp70s interact with the nascent polypeptide at a very early stage of chain elongation. Given the high density of total protein in the cytosol (20–30%) and of unfolded protein molecules (up to 30–50 μM in *E. coli*), this interaction appears to be necessary to prevent the aggregation of nascent chains or their unfavourable association with the ribosome surface at a point when the partially synthesized and therefore conformationally restricted polypeptide is not yet able to form a stable tertiary structure. At a later stage, the not yet folded polypeptide can be transferred to a chaperonin of the Hsp60 family (in bacteria, mitochondria and chloroplasts) or the TCP-1 family (in the eukaryotic cytosol), which mediates folding to the native state (Langer *et al.* 1992; Frydman *et al.* 1994). In the case of the TCP-1 ring complex (TRiC), this transfer can occur co-translationally before completion of synthesis (Frydman *et al.* 1994).

(*a*) *Mechanism of the Hsp70 system*

The Hsp70s have the ability to bind short, extended peptide segments of seven or eight residues which are enriched in hydrophobic amino acids (Flynn *et al.* 1991; Blond-Elguindi *et al.* 1993). Only more recently has it been realized that for full function the Hsp70s

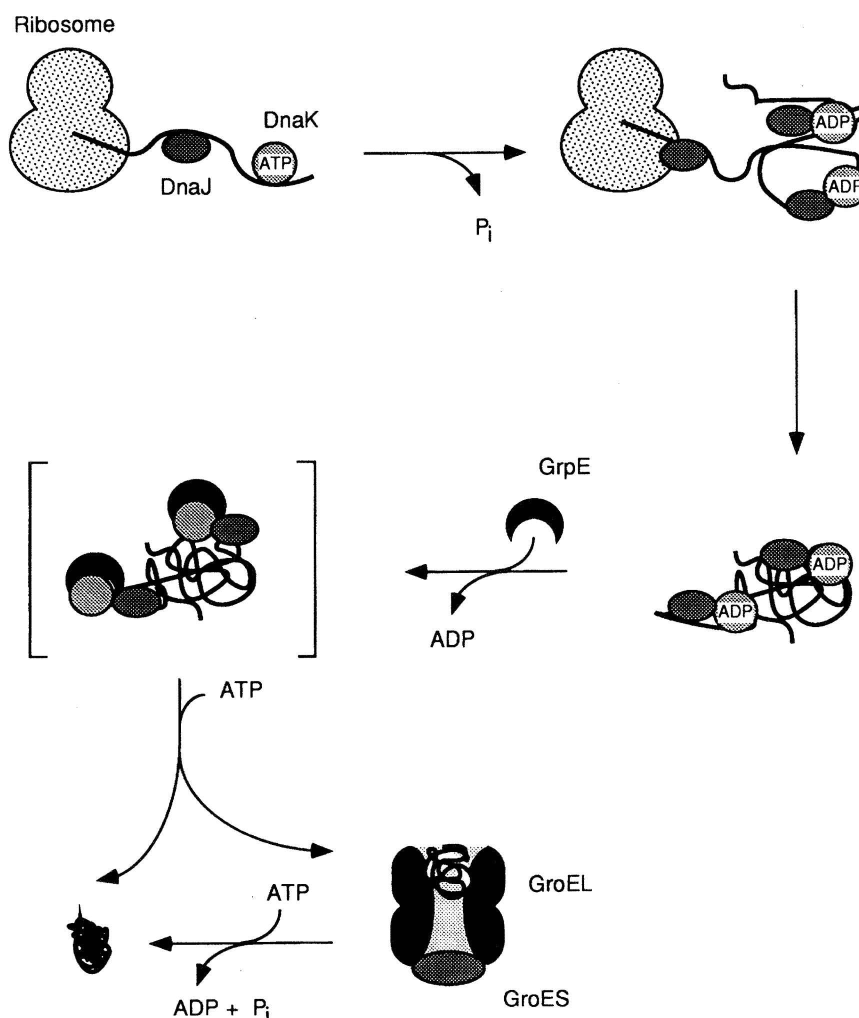

Figure 1. Model for the pathway of protein folding in the *E. coli* cytosol. The polypeptide chain emerging from the ribosome is bound by DnaJ and DnaK (Hsp70) (Hendrick *et al.* 1993; Gaitanaris *et al.* 1994). The direct interaction between DnaK and DnaJ in the presence of ATP leads to the formation of a ternary complex between nascent chain, DnaK and DnaJ in which DnaK is in the ADP-state. This complex dissociates upon the GrpE-dependent dissociation of ADP and the binding (not hydrolysis) of ATP to DnaK (Szabo *et al.* 1994). The protein may then fold to the native state by multiple rounds of interaction with the DnaK, DnaJ, GrpE system or is transferred for final folding to GroEL/GroES (Langer *et al.* 1992*a*). We assume that a large fraction of cytosolic proteins have to interact with both chaperone systems to reach the native state.

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depend on the regulation by further proteins. In *E. coli* the Hsp70, called DnaK, cooperates with the chaperone DnaJ and the nucleotide exchange factor GrpE, proteins of about 43 kDa and 23 kDa, respectively (Georgopoulos 1992). Both DnaK and DnaJ bind nascent polypeptide chains cotranslationally (Hendrick *et al.* 1993; Gaitanaris *et al.* 1994; Kudlicki *et al.* 1994), whereby DnaJ seems to mediate the loading of DnaK onto the elongating chain (figure 1). The interaction of DnaJ with DnaK accelerates the hydrolysis of DnaK-bound ATP to ADP (Liberek *et al.* 1991) and stabilizes the ADP-state of DnaK which has a high affinity for unfolded polypeptide (Palleros *et al.* 1993). As a result, a stable ternary complex consisting of polypeptide substrate, DnaJ and ADP-bound DnaK is formed (Langer *et al.* 1992; Szabo *et al.* 1994). GrpE then functions as a nucleotide exchange factor for DnaK in dissociating the bound ADP, whereupon ATP binding to DnaK causes the release of the polypeptide substrate (Szabo *et al.*, 1994). This allows the transfer of the unfolded protein to GroEL, the bacterial Hsp60 (Langer *et al.* 1992). At least *in vitro*, the folding of certain proteins may be achieved through ATP-dependent cycles of binding and release to DnaK and DnaJ alone (Schröder *et al.*, 1993; Szabo *et al.*, 1994). *In vivo*, however, the primary function of the Hsp70 system seems to be in maintaining the polypeptide chain in a non-aggregated state, competent for folding by the chaperonin. The eukaryotic cytsosol contains several DnaJ homologues (Caplan & Douglas 1993), but a structural or functional equivalent of GrpE has not yet been identified in this compartment.

(*b*) *Mechanism of the chaperonin system*

While the Hsp70 and DnaJ proteins function as monomers or dimers, the chaperonin of *E. coli*, GroEL, is a large complex consisting of two stacked rings of seven identical 60 kDa subunits, forming a cylinder with a central cavity (Hendrix 1979; Hohn *et al.* 1979; Langer *et al.* 1992*b*; Saibil *et al.* 1993; Braig *et al.* 1994). GroEL has an essential cofactor, GroES, a single heptameric ring of 10 kDa subunits that binds to GroEL and increases the cooperativity of ATP hydrolysis in the GroEL ring system. Although this regulation is not required for the ATP-dependent release of bound protein from GroEL per se, with many substrate proteins it is necessary to make the release reaction productive for folding. Under most conditions, binding of GroES to either end of the GroEL cylinder strongly reduces the affinity of the opposite end for binding a second GroES (Langer *et al.* 1992; Saibil *et al.* 1993; Chen *et al.* 1994). This negative cooperativity of GroES binding is decreased at high concentrations of Mg^{2+} (15–50 mM) and at elevated pH (pH 7.7–8.0), conditions which allow the formation of symmetrical GroES:GroEL:GroES complexes (Llorca *et al.* 1994; Schmidt *et al.* 1994). A recent kinetic analysis of the GroEL–GroES reaction cycle using the new technique of surface plasmon resonance (Biacore™) failed to demonstrate the functional significance of these so-called 'football' structures (M.K. Hayer-Hartl, unpublished results).

The interaction between GroEL and GroES is dynamic, both in the absence and presence of substrate polypeptide. The asymmetrical binding of GroES stabilizes the seven subunits of GroEL that are in contact with GroES in a tight ADP state, resulting in a 50% inhibition of the GroEL ATPase (Martin *et al.* 1993; Todd *et al.* 1993). Binding and hydrolysis of ATP in the opposite heptamer of GroEL causes the transient release of the tightly bound ADP and GroES. This cycling of GroES between bound and free states is normally slow but is accelerated by the association of polypeptide substrate with GroEL (Martin *et al.* 1993). Polypeptide binding stimulates the ATPase activity of GroEL (Martin *et al.* 1991; Jackson *et al.* 1993). The unfolded polypeptide binds initially to the GroEL ring that is not covered by GroES (figure 2). This facilitates the release of the tightly bound ADP and the dissociation of GroES. Upon ATP binding, GroES may then reassociate with the protein-containing ring of GroEL, inducing the ATP-hydrolysis-dependent release of the bound polypeptide (Martin *et al.* 1993). Interestingly, binding of GroES causes a massive outwards movement of the apical domains of the GroEL subunits, creating an enclosed, dome-shaped space with a maximum height and width of 70 Å (figure 2) (Chen *et al.* 1994). GroES could initially make contact with the outer surface of the GroEL cylinder, triggering further domain movement, thus transiently displacing the polypeptide substrate into the cavity for folding (Fenton *et al.* 1994; Hartl 1994). At least partial folding may thus occur in a shielded microenvironment, before the polypeptide emerges from the chaperonin cavity (Martin *et al.* 1993). Multiple rounds of binding and release to GroEL may be necessary for completion of folding (Martin *et al.* 1991). Alternatively, GroES may exert its function aiding productive protein release from the GroEL ring that is not occupied by the folding polypeptide. Both mechanisms of GroES action may not be mutually exclusive (figure 2).

GroEL binds its substrate in the conformation of a compact, yet flexible, molten globule-state which exposes hydrophobic surfaces to solvent (Martin *et al.* 1991; Hayer-Hartl *et al.* 1994; Robinson *et al.* 1994). The structure-based mutational analysis of GroEL indeed suggests the presence of a complementary hydrophobic binding surface that lines the cavity of the cylinder (Fenton *et al.* 1994). In contrast to Hsp70, GroEL does not seem to recognize short peptide sequences in extended conformations (Landry *et al.* 1992).

The exact extent of folding that can occur while a polypeptide is in association with the chaperonin, either bound to its surface or upon release into its cavity, remains to be defined. Evidence has been presented that the substrate polypeptide is released into the bulk solution in a conformation that is significantly less prone to aggregation than the conformation initially bound by the chaperonin (Martin *et al.* 1991). While small, single-domain proteins, such as barnase, may reach their native state in association with GroEL (Gray & Fersht 1993), other proteins can be released before they have reached the native state

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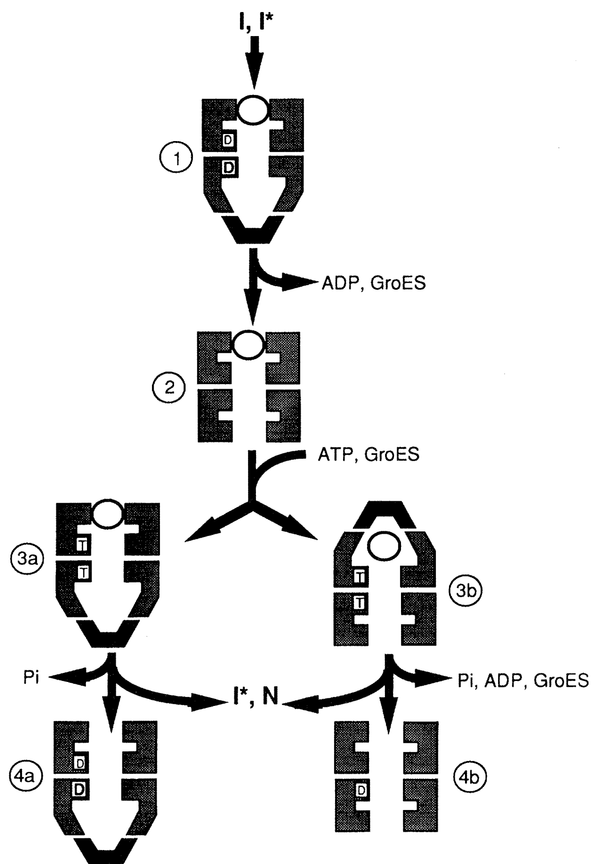

Figure 2. Model for the ATP-dependent interaction between GroEL, GroES and folding polypeptide. D (bold), the high-affinity ADP state in the seven subunits of GroEL which are bound to GroES; D (not bold), the lower ADP affinity of the subunits in the opposite toroid which may hydrolyse ATP (Martin *et al.* 1993); T, the subunits in a GroEL toroid in the ATP-bound state; I, polypeptide substrate as compact folding intermediate; I*, folding intermediate part way advanced towards the native state (for polypeptides with separate domains or subdomains); N, native protein. 1, Polypeptide binding facilitates dissociation of tightly bound ADP and GroES. 2, Polypeptide transiently bound in nucleotide-free toroid. 3, ATP and GroES rebind. GroES associates either with free GroEL ring (3a) or with polypeptide-containing ring (3b). ATP-hydrolysis leads to polypeptide release (4a) and incompletely folded polypeptide rebinds in (1). In 3b, polypeptide is transiently enclosed in the central cavity and is free to fold. ATP-hydrolysis in the GroES-bound toroid then generates the tight ADP-state (not shown). ADP dissociates upon ATP-hydrolysis in the opposite ring, causing dissociation of GroES and allowing polypeptide release. Polypeptide may rebind in (2).

and subsequently re-bind to the chaperonin (Martin *et al.* 1991; Weissman *et al.* 1994).

3. PERSPECTIVES

The functional cooperation between molecular chaperones in protein folding is emerging as a common theme from a number of recent studies (Hendrick & Hartl 1993; Hartl *et al.* 1994). This applies also to the endoplasmic reticulum, the compartment that is responsible for the folding and assembly of secretory proteins. During translocation across the endoplasmic reticulum (ER) membrane, secretory proteins probably interact first with the Hsp70 homologue BiP, followed by interactions with various chaperones (Helenius *et al.* 1992), including the membrane-bound protein calnexin (Bergeron *et al.* 1994). Unlike the cytosol, the environment of the ER lumen is oxidizing and contains the enzyme protein disulphide isomerase which accelerates the correct formation of disulphide bonds (Freedman 1989). A ring-shaped chaperonin is apparently absent from the ER.

With respect to the protein folding problem, traditionally the domain of biophysicists and theoreticians, it will be interesting to see whether molecular chaperones may be able to influence the pathways of protein folding or even the final outcome of a folding reaction. Are there situations where the information specified in the linear sequence of amino acid residues is not sufficient for folding to the native state? Has the coevolution of proteins and chaperones perhaps favoured certain folding pathways over others? To address these questions, the conformational dynamics of chaperone-substrate protein interactions will have to be resolved.

Another important direction of research will be the analysis of protein folding in the context with translation. Very little is known about the very early events of folding that may occur when the growing polypeptide chain is still within the ribosomal exit tunnel or groove. To what extent does the formation of secondary and tertiary structure proceed co-translationally? Here the final goal would be the *in vitro* reconstitution of translation and folding of a nascent polypeptide chain with all the necessary components in purified form.

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