Mini-Review

Chaperonin-mediated Folding of Actin and Tubulin

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THE central dogma of molecular biology holds that proteins contain within their primary amino acid sequence all the information that is required to dictate their three-dimensional structure. In principle, therefore, proteins can fold spontaneously (2). It is now clear, however, that the proper folding of many proteins requires facilitation. The facilitation reaction is accomplished via interaction with chaperonins, a class of multisubunit toroidal complexes which hydrolyzes ATP as part of the mechanism whereby its members contribute to the productive folding of their target polypeptides (4, 5, 11, 12, 15, 16, 20). Chaperonins are both structurally and functionally distinct from a class of molecules (typified by the heat shock protein hsp70) that is also thought to participate in the overall protein folding pathway by maintaining polypeptides in a partially unfolded conformation pending their translocation or presentation to chaperonin (for a review see reference 15). Bacteria, chloroplasts, mitochondria, and the cytosol of eukaryotes each contain a single kind of chaperonin molecule. These are different from one other, but homologous. No chaperonin has so far been found in the endoplasmic reticulum. Here we present our view of how the eukaryotic cytosolic chaperonin functions, particularly in relation to what is known about the mechanism of facilitated folding by chaperonin from prokaryotes.

The Jack-in-the-Box Model

Much of what we know about chaperonin-mediated protein folding has been derived from extensive studies on the *E. coli* paradigm, GroEL. In common with other chaperonins, GroEL is a toroidal structure; each of its two stacked rings is assembled from seven identical 60 kD subunits (5). In conjunction with the cochaperonin GroES—itself a single seven-membered ring of 10 kD subunits (6) that binds to GroEL and coordinates its ATPase activity (19, 27, 28)—GroEL facilitates the folding of many *E. coli* proteins (17, 30).

The mechanism whereby GroEL/GroES facilitates protein folding was originally thought to involve sequestration of the bound target protein, providing a protected environment in which spontaneous folding could occur in the absence of intermolecular interactions that would otherwise lead to aggregation (the Jack-in-the-Box model) (1, 9, 11). Electron microscopic evidence (7, 19), and, more recently, crystallographic analysis (5) coupled with a study of site directed mutants that fail to bind target protein (10) point to the inside rim of the chaperonin as the site where target proteins bind, presumably via exposed hydrophobic surfaces. Upon ADP/ATP exchange and hydrolysis, the chaperonin was thought to release its bound polypeptide into the central cavity, giving the opportunity for correct folding to occur; any off-pathway reactions would result in rebinding, so that the native structure would eventually form as a result of sufficient rounds of ATP-dependent chaperonin-target protein interaction. Ultimately, since hydrophobic surfaces are typically buried in native proteins, the correctly folded protein would no longer bind to the chaperonin, and would be discharged into free solution (Fig. 1, cycle *a*).

Cytosolic Chaperonin and the Cycling of Target Proteins

Cytosolic chaperonin is the functional homolog of GroEL in the cytosol of eukaryotes (11, 12, 24). Unlike GroEL, to which it is only distantly related, cytosolic chaperonin is assembled from eight different (though homologous) polypeptides (18, 23). Both biochemical (11, 12, 24) and genetic studies (8, 29, 31) implicate the participation of cytosolic chaperonin in the facilitated folding of actin and tubulin, as well as actin and tubulin-related polypeptides (21). In the case of actin, the action of cytosolic chaperonin alone in the presence of ATP is sufficient for the generation of native product (12). In contrast, the facilitated folding of the GTP-binding proteins α- and β-tubulin requires cytosolic chaperonin, ATP, GTP, and additional protein cofactors (3, 13, 23). The mechanism of action of protein cofactors in the productive folding of α- and β-tubulin has yet to be established in detail. However, when a cytosolic chaperonin-mediated α- or β-tubulin folding reaction is done in the presence of ATP and GTP but without cofactors, the target protein is released in a nonnative form that can be captured by other chaperonin molecules: for example, the inclusion of excess mitochondrial chaperonin in such reactions results in the bulk transfer of labeled tubulin target protein to mitochondrial chaperonin (14).

The ATP-dependent discharge of nonnative target proteins is not peculiar to the facilitated folding of tubulin: in the case of GroEL, both isotope dilution experiments (28) and the use of mutant GroEL molecules that can act as traps for the capture of nonnative molecules generated

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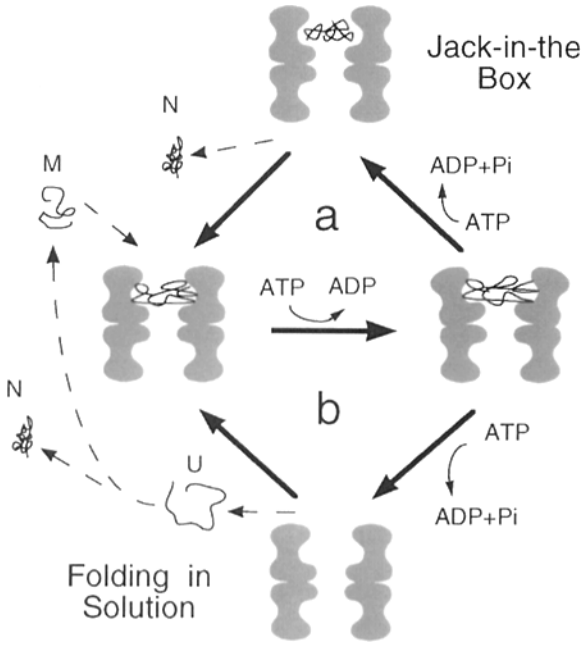

Figure 1. Models of chaperonin action. Solid arrows depict the chaperonin cycle; dashed arrows show the entry or exit of target protein. Unfolded or misfolded target protein (*M*) binds within the chaperonin's central cavity. The exchange of ATP for ADP results in a conformational change in the chaperonin (depicted as a tilting of shaded chaperonin subunits). In the Jack-in-the-Box model (cycle *a*), ATP hydrolysis results in the release of target protein into the chaperonin's central cavity. Target protein then either rebinds to chaperonin to begin a new cycle, or, if it reaches the native state (*N*), it is released into solution. In the Folding-in-Solution model (cycle *b*), ATP hydrolysis results in the release of target protein into bulk solution as unfolded intermediates (*U*) which undergo kinetic partitioning, either folding spontaneously to the native state, or again misfolding and rebinding to another chaperonin molecule to begin a new cycle of interaction with chaperonin.

during facilitated folding (32) point to a similar mechanism in which nonnative proteins are "cycled" among different chaperonin molecules before partitioning to the native state.

Chaperonins as Unfolders of Off-Pathway Intermediates

The demonstration of the phenomenon of target protein cycling has prompted a rethinking of how chaperonins might work. Since target molecules can be discharged in a nonnative state, an attractive idea seemed to be that the chaperonin functioned by unfolding target molecules that were kinetically trapped, and discharging these unfolded intermediates so that they could fold spontaneously in bulk solution. Molecules that fail to partition to the native state and again become kinetically trapped would then rebind to another chaperonin molecule, become unfolded once more, and be released into solution where they would "try again" to reach the native state (28, 32) (Fig. 1, cycle *b*). This concept of chaperonin action is consistent with the notion that the chaperonin itself imparts no information to the target molecule—it merely unfolds it, without discriminating between native and nonnative contacts—and also with the well-established principle that target proteins themselves are endowed with all the necessary information required for them to reach the native state (2).

Chaperonin Specificity

If, as the above model suggests, the function of chaperonins is to act as unfolders of kinetically trapped intermediates, then it seems reasonable to suppose that chaperonins would operate indiscriminately, facilitating the folding of whatever misfolded target proteins they might encounter. This notion is consistent with the observation that GroEL facilitates the folding of a range of target proteins in *E. coli* (17), while cytosolic chaperonin, which behaves as a single functional molecular species, facilitates the folding of β-actin, the vertebrate actin-related protein actin-RPV, and α-, β-, and γ-tubulin (21). However, experimental evidence shows that chaperonins cannot indiscriminately facilitate the folding of any target protein (25). When β-actin is presented to GroEL as an unfolded target protein, for example, a binary complex is formed; incubation with ATP results in the cycling of β-actin between GroEL molecules, but not in the production of correctly folded protein. Similarly, tubulin target proteins bound to GroEL do not reach the native state upon incubation with ATP and GTP, whether or not the appropriate cofactors are included in the reaction. These experiments demonstrate that, while GroEL is capable of the ATP-dependent cycling of actin and tubulin, the nonnative forms of target protein released by the prokaryotic chaperonin at each cycle cannot partition to the native state. Thus, distinct spectra of intermediates are produced by different kinds of chaperonin (25).

Stable Intermediates in Tubulin Folding

Clearly, a detailed understanding of the mechanism of chaperonin action depends on an analysis of the intermediates generated during folding reactions. This presents a major experimental challenge, since such intermediates are not only vastly heterogeneous, but also transient in existence and very prone to aggregation. Fortunately, the fact that cytosolic chaperonin-mediated tubulin folding reactions require the participation of protein cofactors presents a unique opportunity to study such intermediates, because in tubulin folding reactions, the action of cytosolic chaperonin (which is ATP dependent) can be uncoupled from the action of cofactors (which is ATP independent): the yield of native tubulin produced in in vitro folding reactions is essentially the same whether cofactors are present at the outset, or added after incubation with ATP and GTP and quenching of the ATP-dependent reaction with hexokinase and glucose. Thus, in the absence of cofactors, α-tubulin folding intermediates accumulate (26).

The α-tubulin folding intermediates that accumulate during cytosolic chaperonin-mediated folding are fairly stable, with a half-life of 50 min at 30°, and are the substrates upon which cofactors act in order to generate native tubulin. These intermediates, defined as Intermediates, Quasifolded (I_Q)[1], are chaperonin-associated, since

1. *Abbreviations used in this paper*: I_Q, Intermediates Quasifolded.

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they comigrate with cytosolic chaperonin on sucrose gradients and on gel filtration columns. Moreover, though most α-tubulin intermediates do cycle in the cytosolic chaperonin-mediated folding reaction, I_Q intermediates do not: thus, I_Q intermediates represent an end-state of cytosolic chaperonin-bound molecules that require cofactors to extricate them from a terminal kinetic trap.

Two lines of evidence show that α-tubulin I_Q intermediates are extensively folded. First, they are more resistant to proteolysis than intermediates formed by presentation of target protein to cytosolic chaperonin without incubation with ATP. Second, they contain nonexchangeably bound GTP, demonstrating that the GTP-binding pocket is in its native configuration. Since some I_Q molecules are formed via a single cycle of interaction of target protein with cytosolic chaperonin, it follows that folding of α-tubulin molecules to the quasifolded state can occur while the target protein is cytosolic chaperonin bound (26).

Target Range of Cytosolic Chaperonin

If actin and tubulin folding occurs in part on the surface of cytosolic chaperonin, then the spectra of folding intermediates released would be determined by the details of the interaction between the cytosolic chaperonin and these two classes of target protein. Such interactions could reflect the evolution of cytosolic chaperonin in eukaryotes as a chaperonin specifically tailored for the facilitated folding of actins and tubulins. Note, however, that this concept does not entail any informational input from cytosolic chaperonin into the final configuration of target protein; rather, it could reflect cytosolic chaperonin's ability to overcome kinetic traps that are specific to tubulin and actin folding pathways. Several lines of evidence suggest that, unlike GroEL, the target range of cytosolic chaperonin is highly restricted. First, cytosolic chaperonin has a much higher affinity for actin and tubulin target proteins compared with other cytosolic proteins of noncytoskeletal origin (22). Second, when cytosolic chaperonin binary complexes are isolated from cells pulse labeled with [^{35}S]methionine, actins and tubulins constitute the overwhelming majority of detectable cytosolic chaperonin-associated proteins (reference 24; Vainberg, I.E., and N.J. Cowan, unpublished observations). Third, a tissue such as liver, which is committed to the abundant synthesis of a wide spectrum of cytosolic proteins, but synthesizes only a relatively modest level of actins and tubulins, contains a correspondingly low level of cytosolic chaperonin (14). Finally, mutations in the constituent polypeptides of cytosolic chaperonin result in exclusively cytoskeletal phenotypes (8, 29, 31).

A Model for Chaperonin Action

How might we envision the mechanism of cytosolic chaperonin action? There is evidence that folding intermediates bind to ADP/cytosolic chaperonin (22), presumably via exposed hydrophobic surfaces. ADP/ATP exchange then occurs, resulting in an altered conformation of chaperonin (7, 12) (Fig. 1). We propose that this combination of binding and flexing by cytosolic chaperonin causes a conformational change in bound actin or tubulin target proteins ("tweaking"), potentially lifting them over kinetic barriers to productive folding. By analogy with GroEL (28, 32), ATP hydrolysis then results in the release of these target proteins, which either reach the native state or become kinetically trapped and are recaptured by other cytosolic chaperonin molecules. This is essentially the Folding-in-Solution model depicted in Fig. 1, cycle *b*. However, given the existence of I_Q intermediates and the target protein specificity of chaperonins, it is clear that the spectrum of states of target protein released from chaperonin (U in Fig. 1) must include intermediates that are far from fully unfolded, and that have acquired their native-like structure while bound to chaperonin.

Because of space limitations, we apologize to those whose work was not directly cited. The term "Jack-in-the-Box" as applied to certain models of chaperonin-mediated folding was originally coined by Dr. George Lorimer.

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References

1. Agard, D. A. 1993. To fold or not to fold. *Science (Wash. DC).* 260:1903–1904.
2. Anfinsen, C. B. 1973. Principles that govern the folding of protein chains. *Science (Wash. DC).* 181:223–230.
3. Archer, J. E., L. R. Vega, and F. Solomon. 1995. Rbl2, a yeast protein that binds to β-tubulin and participates in microtubule function in vivo. *Cell.* 82:425–434.
4. Bochkareva, E. S., N. M. Lissin, and A. S. Girshovich. 1988. Transient association of newly synthesized unfolded proteins with the heat shock GroEL protein. *Nature (Lond.).* 336:254–257.
5. Braig, K., Z. Otwinowski, R. Hegde, D. Boisvert, A. Joachimiak, A. L. Horwich, and P. B. Sigler. 1994. The crystal structure of the bacterial chaperonin GroEL at 2.8A. *Nature (Lond.).* 578–586.
6. Chandrasekhar, G. N., K. Tilly, C. Woolford, R. Hendrix, and C. Georgopoulos. 1986. Purification and properties of the groES morphogenetic protein of *Escherichia coli. J. Biol. Chem.* 261:12414–12419.
7. Chen, S., A. M. Roseman, A. S. Hunter, S. P. Wood, S. G. Burston, N. A. Ranson, A. R. Clarke, and H. R. Saibil. 1994. Location of a folding protein and shape changes in GroEL-GroES complexes imaged by cryo-electron microscopy. *Nature (Lond.).* 371:261–264.
8. Chen, X., D. S. Sullivan, and T. Huffaker. 1994. Two yeast genes with similarity to TCP-1 are required for microtubule and actin function in vivo. *Proc. Natl. Acad. Sci. USA.* 91:9111–9115.
9. Ellis, R. J. 1993. Chaperonin duet. *Nature (Lond.).* 366:213–214.
10. Fenton, W. A., Y. Kashi, K. Furtak, and A. L. Horwich. 1994. Residues in chaperonin GroEL required for polypeptide binding and release. *Nature (Lond.).* 371:614–619.
11. Frydman, J., E. Nimmesgern, H. Erdjument-Bromage, J. S. Wall, P. Tempst, and F-U. Hartl. 1992. Function in protein folding of TRiC, a cytosolic ring complex containing TCP-1 and structurally related subunits. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:4767–4778.
12. Gao, Y., J. O. Thomas, R. L. Chow, G-H. Lee, and N. J. Cowan. 1992. A cytoplasmic chaperonin that catalyzes β-actin folding. *Cell.* 69:1943–1050.
13. Gao, Y., I. E. Vainberg, R. L. Chow, and N. J. Cowan. 1993. Two cofactors and cytoplasmic chaperonin are required for the folding of α- and β-tubulin. *Mol. Cell Biol.* 13:2478–2485.
14. Gao, Y., R. Melki, P. D. Walden, S. A. Lewis, C. Ampe, H. Rommelaere, J. Vandekerckhove, and N. J. Cowan. 1994. A novel cochaperonin that modulates the ATPase activity of cytoplasmic chaperonin. *J. Cell Biol.* 125:989–996.
15. Gething, M.-J., and J. Sambrook. 1992. Protein folding in the cell. *Nature (Lond.).* 355:33–44.
16. Goloubinoff, P., J. T. Christeller, A. A. Gatenby, and G. H. Lorimer. 1989. Reconstitution of active ribulose biphosphate carboxylase from an unfolded state depends on two chaperonin proteins and Mg-ATP. *Nature (Lond.).* 342:884–889.
17. Horwich, A. L., K. Brooks Low, W. A. Fenton, I. N. Hirshfield, and K. Furtak. 1993. Folding in vivo of bacterial proteins: role of GroEL. *Cell.* 74: 909–917.
18. Kubota, H., G. Hynes, A. Carne, A. Ashworth, and K. Willison. 1994. Identification of six Tcp-1-related genes encoding divergent subunits of the TCP-1-containing chaperonin. *Curr. Biol.* 4:89–99.
19. Langer, T., G. Pfeifer, J. Martin, W. Baumeister, and F.-U. Hartl. 1992. Chaperonin-mediated protein folding: GroES binds to one end of the GroEL cylinder which accommodates the protein substrate within its

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central cavity. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:4757–4765.
20. Martin, J., T. Langer, R. Boteva, A. Schramel, A. L. Horwich, and F.-U. Hartl. 1991. Chaperonin-mediated folding at the surface of GroEL through a 'molten-globule-like' intermediate. *Nature (Lond.).* 352:36–42.
21. Melki, R., I. E. Vainberg, R. L. Chow, and N. J. Cowan. 1993. Chaperonin-mediated folding of vertebrate actin-related protein and γ-tubulin. *J. Cell Biol.* 122:1301–1310.
22. Melki, R., and N. J. Cowan. 1994. Facilitated folding of actins and tubulins occurs via a nucleotide-dependent interaction between cytoplasmic chaperonin and distinctive folding intermediates. *Mol. Cell Biol.* 14:2895–2904.
23. Rommelaere, H., M. Van Troys, Y. Gao, R. Melki, N. J. Cowan, J. Vandekerckhove, and C. Ampe. 1993. The eukaryotic cytosolic chaperonin contains t-complex polypeptide 1 and seven related subunits. *Proc. Natl. Acad. Sci. USA.* 90:11975–11979.
24. Sternlicht, H., G.W. Farr, M. Sternlicht, J. K. Driscoll, K. Willison, and M. Yaffe. 1993. The t-complex polypeptide 1 complex is a chaperonin for tubulin and actin in vivo. *Proc. Natl. Acad. Sci. USA.* 90:9422–9426.
25. Tian, G., I. E. Vainberg, W. D. Tap, S. A. Lewis, and N. J. Cowan. 1995. Specificity in chaperonin-mediated protein folding. *Nature (Lond.).* 375: 250–253.
26. Tian, G., I. E. Vainberg, W. D. Tap, S. A. Lewis, and N. J. Cowan. 1995. Quasi-native intermediates in chaperonin-mediated protein folding. *J. Biol. Chem.* 270:23910–23913.
27. Todd, J. M., P. V. Viitanen, and G. H. Lorimer. 1993. Hydrolysis of adenosine 5′-triphosphate by *Escherichia coli* GroEL: effects of GroES and potassium ion. *Biochemistry.* 32:8560–8567.
28. Todd, J. M., P. V. Viitanen, and G. H. Lorimer. 1994. Dynamics of the chaperonin ATPase cycle: implications for facilitated protein folding. *Science (Wash. DC).* 265:659–666.
29. Ursic, D., and M. R. Culbertson. 1991. The yeast homolog to mouse TCP-1 affects microtubule-mediated processes. *Mol. Cell Biol.* 11:2629–2640.
30. Viitanen, P. V., A. A. Gatenby, and G. H. Lorimer. 1992. Purified chaperonin 60 (groEL) interacts with nonnative states of a multitude of *Escherichia coli* proteins. *Protein Sci.* 1:363–369.
31. Vinh, D. B-N., and D. G. Drubin. 1994. A yeast TCP-1-like protein is required for actin function in vivo. *Proc. Natl. Acad. Sci. USA.* 91:9116–9120.
32. Weissman, J. S., Y. Kashi, W. A. Fenton, and A. L. Horwich. 1994. GroEL-mediated protein folding proceeds by multiple rounds of binding and release of non-native forms. *Cell.* 78:693–702.

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