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Commentary

Dissecting intrinsic chaperonin activity

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It has long been known that the linear sequence of amino acids along the polypeptide chains contains all the necessary information required to determine the correct three-dimensional structure of a protein (1). Indeed, a large number of proteins have been shown to refold spontaneously *in vitro* from an unfolded denatured state to the native folded state (2–6). Other proteins, often large ones, however, do not refold spontaneously in high yields. This is usually due to irreversible aggregation of unfolded or partially folded states (7–9). Such proteins require the presence of ancillary proteins known as chaperonins to assist in the folding process (10–13). The two best studied chaperonins are the *Escherichia coli* GroEL and GroES chaperonins which act in concert (for reviews, see refs. 14–18). GroEL has a cage-like double ring structure with two internal cavities, each ring comprising seven identical (≈15 kDa) subunits; GroES caps one end of the GroEL double ring and consists of a single ring of seven (≈10 kDa) identical subunits (19–21). *In vivo* intact GroEL, GroES, and ATP are required for function (14–18, 22, 23). There are several features of the chaperonins that have been considered a key to their activity: (*i*) the ability to unfold incorrectly folded, kinetically trapped intermediates, thereby permitting another attempt at correct refolding to take place, a process akin to iterative annealing (24, 25); (*ii*) the presence of a central cavity in which proteins can refold in isolation (7, 26–30); and (*iii*) the allosteric switching of GroEL from a state with high affinity for unfolded or partially folded peptides to one with low affinity upon binding ATP and GroES (30–33). Each subunit of GroEL consists of three domains (18, 19, 34, 35): an equatorial domain which contains the nucleotide binding site and which provides most of the intersubunit contacts; an apical domain which binds both peptides and GroES; and a linker domain which serves to transmit allosteric effects between the two other domains. In a landmark paper recently published in the *Proceedings*, Fersht and colleagues (36) now demonstrate that a monomeric version of the apical domain can assist protein folding in the absence of nucleotides, GroES, and, of course, the central cavity.

Zahn *et al.* (36) expressed two constructs of the apical domain, comprising residues 191–376 and 191–345, with and without the two C-terminal helices, respectively. The x-ray structure of the shorter construct was solved and has the same fold as that of native GroEL. Zahn *et al.* (36) tested the chaperonin activity of the two apical domain constructs in three separate *in vitro* assays: (*i*) unfolding of barnase, (*ii*) refolding of cyclophilin A, and (*iii*) refolding of rhodanese. Both cyclophilin A and rhodanese require chaperonins to refold at high yield *in vitro*. In all three assays, the apical domain constructs displayed features that mimic some of the activities of intact GroEL. The apical domain binds tightly to unfolded barnase, albeit with an ≈5-fold lower affinity. In the case of cyclophilin A, both the rate and yield of refolding in the presence of the apical domain is the same as that obtained by GroEL. Finally, the rate of rhodanese refolding in the presence of either the apical domain or GroEL is essentially the same, although the yield with the apical domain constructs (≈55% and ≈40% for the short and long constructs, respectively), is a factor of about two lower than that with GroEL, GroES, and ATP (≈90%). Interestingly, the refolding yield obtained with the apical domain is reduced by the addition of GroES, presumably because the peptide and GroES binding sites on the apical domain partially overlap.

What is the mechanism of intrinsic chaperonin activity? From a simplistic viewpoint, one can envisage chaperonin-assisted folding as follows. In the absence of chaperonin, there is competition between the folding and aggregation pathways. Providing the folding process to either the native state or, alternatively, a collapsed nonaggregating state (which is competent to undergo spontaneous refolding), is significantly faster that the aggregation rate, a high yield of native folded protein will be obtained. If, on the other hand, the aggregation pathway is dominant, the refolding yield will be low. In such cases, the presence of chaperonin can increase the refolding yield by binding reversibly to a wide variety of unfolded states. This interaction can be of a relatively nonspecific nature involving contact between exposed hydrophobic surfaces of the apical domain and the unfolded polypeptide. [In this light, it is interesting to note that even BSA has weak chaperonin activity and increases the refolding yield of rhodanese by a factor of about two, from ≈5% in its absence to ≈10% in its presence, presumably by binding in a nonspecific manner to the unfolded polypeptide (36).] Thus, self-association of aggregation prone unfolded states is prevented, permitting a subsequent attempt at correct folding to occur upon dissociation of the polypeptide in an initially nonaggregating unfolded form. Hence chaperonins act as kinetic proofreaders, removing undesired aggregation prone states along the protein folding pathway (37, 38).

The results presented by Zahn *et al.* (36) demonstrate that the monomeric apical domain possesses intrinsic chaperone activity, similar to that of the intact GroEL/GroES/ATP system, and hence can be considered to represent a mini-chaperone. What are the implications of this finding with respect to the role of allostery and the central cavity? *In vitro*, the affinity of the monomeric apical domains for substrate is high enough for chaperonin activity but weak enough to permit dissociation and subsequent refolding to occur (36). In the *in vivo* situation, however, GroES serves to modulate substrate binding affinity, thereby permitting GroEL to initially bind and unfold a large variety of incorrectly folded substrates, followed by their subsequent release to permit folding to occur (14–18, 33, 36). The cavity also plays an important role *in vivo* by ensuring that folding of unfolded polypeptide occurs in isolation. By this means, folding of unfolded states can occur in effect at infinite dilution because, for reasonably large proteins, there is room for only one polypeptide within the cavity, preventing both self-association and aggregation with other cellular components, and thereby increasing the refolding yield. In this regard, it is worth noting that *in vivo* a difference in yield of a factor of two (as was observed, for

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example, for rhodanese refolding in the presence of intact GroEL/GroES/ATP versus isolated apical domain) can make all the difference between the presence or absence of a particular trait or phenotype.

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