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Chaperone machines in action

Helen R Saibil

How do chaperones operate in cells? For some major chaperones it is clear what they do, though mostly not how they do it. Hsp60, 70 and 100 families carry out folding, unfolding or disaggregation of proteins. Regarding mechanisms of action, we have the clearest picture of the ATP-driven mechanism of the bacterial Hsp60s, and structures of full-length Hsp70 and 90 family members are beginning to give insights into their allosteric mechanisms. Recent advances are giving an improved understanding of the nature of chaperone interactions with their non-native substrate proteins. There have also been significant advances in understanding the engagement of chaperones in preventing the formation of toxic aggregates in degenerative disease and the relationship of protein quality control to complex biological processes such as ageing.

Addresses
Department of Crystallography and Institute of Structural Molecular Biology, Birkbeck College, Malet Street, London WC1E 7HX, UK

Corresponding author: Saibil, Helen R (h.saibil@mail.cryst.bbk.ac.uk)

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Introduction

Molecular chaperones orchestrate when and where proteins fold and unfold in the cell, and if there is serious misfolding and aggregation caused by environmental stress or pathology, they can act as sensors to direct the cell to apoptosis. The general chaperones achieve this without stereo-specific information on the structures of their substrates and can cooperate and substitute for each other despite their diversity of operating mechanisms. Failure of correct folding is deleterious, not only because of loss of function of the misfolded species, but also more seriously because of the toxicity of protein aggregates. Common features of chaperone action are transient interaction with non-native species in the prevention of aggregation and promotion of correct folding and assembly, or in unfolding for translocation or targeting to proteases [1–3]. General chaperones, which are typically heat shock proteins, are abundant in the cytosol, ER (endoplasmic reticulum), mitochondria and chloroplasts. This review will mainly focus on the general, ATP-dependent chaperones, particularly on recent structural and functional advances giving new insights into their modes of action.

Protein folding by Hsp60/chaperonins

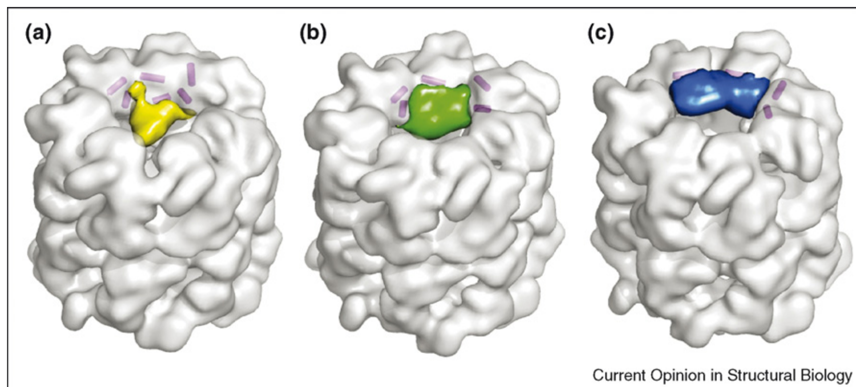
Chaperonins are cylindrical barrel shaped stacked ring complexes with end cavities that alternately bind and encapsulate folding proteins [4]. How are chaperonins able to direct the correct folding of a wide variety of unrelated polypeptides both from nascent chains and from the denatured state after cell stress?

There has been gradual progress on understanding the intricate mechanism of how binding and encapsulation by GroEL and GroES assists the folding of protein subunits. The general idea is that non-native polypeptides are captured by binding to three to four of the seven hydrophobic sites on an open ring of GroEL [5]. Binding of ATP and GroES to a GroEL ring induces major conformational changes that both sequester the binding sites and create an expanded, closed cavity, thus trapping the substrate polypeptide in a hydrophilic chamber for protein folding [4]. Lorimer and colleagues proposed a mechanism of forced unfolding in which collapsed, misfolded proteins are pulled apart by interaction with multiple hydrophobic sites on GroEL that then undergo substantial displacements (reviewed in reference [6]). Key features of the interaction of non-native proteins with GroEL are isolation (prevention of aggregation), unfolding (passive, possibly active to release kinetically trapped states) and confinement [7] (acceleration of folding inside the GroEL–GroES cavity due to restriction of conformational space).

Rapid kinetics studies show multiple steps in the GroEL ATPase cycle, with four distinct transitions in GroEL upon binding of ATP but before hydrolysis, and four further steps associated with GroES binding [8]. Distortions of the inter-ring interface between ATP and ADP states of the GroEL–GroES complex have been observed by cryo EM [9]. A further challenge is to structurally define all these intermediate states, most of which are short lived, by a combination of different approaches. Notably the first transition after GroES binding occurs before release of bound substrate from the hydrophobic binding sites, suggesting a mechanism for efficient encapsulation of the substrate.

Several recent studies report advances in understanding the interaction of non-native substrates with chaperonins. A cryo EM study reports the structure of a set of GroEL–MDH complexes (Figure 1) [10••]. The maps reveal that

Figure 1



GroEL–substrate complexes. Models of three different classes of GroEL complexes with non-native malate dehydrogenase (MDH) are shown with the GroEL surface (grey) modelled from subunit domains fitted to the original maps [10••] and the MDH difference density in different colours. The MDH density in **(a and b)** is located in a deeper position in the cavity, leaving access for GroES to bind the helix H/I binding site (magenta cylinders). The complex in **(c)** has a more extended, two-domain density for MDH bound in a more external position.

although it is disordered, the non-native protein is nevertheless seen in several states, preferentially binding deep inside the cavity (Figure 1a and b), leaving space for GroES to bind without direct competition. In addition, a subset of complexes appears to show a two-domain, molten globule-like state of the substrate (Figure 1c). The bound substrate distorts the GroEL ring so that the apical domains are displaced from their sevenfold symmetric positions. This distortion is transmitted to the opposite ring and may be important in negative cooperativity.

Attempts to understand the folding and assembly of the chloroplast enzyme Ribulose bisphosphate carboxylase oxygenase (Rubisco) stimulated the initial breakthrough in understanding the role of chaperonins [11]. A major recent advance is the characterization of the cyanobacterial form of the chaperonin cofactor RbcX that is required downstream of the chaperonin to stabilize the Rubisco large subunit for assembly into the final 16-mer of large and small subunits [12•]. RbcX dimers provide a binding site for a surface-exposed C-terminal peptide of Rubisco large subunit, preventing aggregation during the assembly process.

Understanding of the eukaryotic cytosolic group 2 chaperonin CCT (TRiC) has lagged behind that of group 1 *E. coli* GroEL. Unlike most chaperones, CCT is not overexpressed during cell stress. It has one-tenth the abundance of GroEL and is far more complex because of its heteromeric ring structure containing 8 different gene products. CCT does not contain obvious hydrophobic binding sites like the group 1 chaperones. It appears to be less general in its specificity but is required for diverse substrates including actin, tubulin, VHL and β-propeller proteins such as transducin-β. Nevertheless, recent studies point to a hydrophobic mode of substrate interaction, possibly analogous to that of GroEL [13•,14•].

Allosteric communication in Hsp70

The abundant and widespread chaperone Hsp70, and its constitutive form Hsc70, consists of two domains, an N-terminal ATPase (nucleotide binding, NBD) domain homologous to actin and hexokinase (Figure 2a) and a C-terminal substrate binding domain (SBD) that binds extended polypeptide chains in a cleft that becomes closed by a helical lid (Figure 2b). The biological roles of Hsp70 family members are wide ranging, both in folding and unfolding for translocation or after disaggregation, signalling heat shock, and as motor for translocation [2]. As for chaperonins, binding and release of substrate are controlled by an ATPase cycle, in cooperation with the cofactor Hsp40 and nucleotide exchange factors (NEFs). Understanding of the interaction between ATPase and substrate binding domains has lagged far behind the knowledge of the individual Hsp70 domain structures. Recent structural studies have addressed the essential allosteric interaction between the NBD and SBD and there are now two crystal structures of intact or nearly intact Hsp70s, one of bovine Hsc70 in a nucleotide-free state [15] and the other, of a more distantly related Hsp70 family member, Hsp110, in the ATP-bound state [16••]. Surprisingly, Hsp110 acts as a NEF for canonical Hsp70s [17,18]. There is a great diversity of NEF mechanisms, with different binding sites around the surface of the NBD that all open the nucleotide cleft in different ways [2], reminiscent of the wide variety of binding partners for actin.

Illustrating the difficulty of studying Hsp70 allostery, the two full-length crystal structures show quite different interfaces between the two domains (Figure 2d and e).

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