Invited Review
ATP-Driven Molecular Chaperone Machines

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ABSTRACT:

This review is focused on the mechanisms by which ATP binding and hydrolysis drive chaperone machines assisting protein folding and unfolding. A survey of the key, general chaperone systems Hsp70 and Hsp90, and the unfoldase Hsp100 is followed by a focus on the Hsp60 chaperonin machine which is understood in most detail. Cryo-electron microscopy analysis of the E. coli *Hsp60 GroEL reveals intermediate conformations in the ATPase cycle and in substrate folding. These structures suggest a mechanism by which GroEL can forcefully unfold and then encapsulate substrates for subsequent folding in isolation from all other binding surfaces. © 2013 Wiley Periodicals, Inc. Biopolymers 99: 846–859, 2013.*

Keywords: chaperones; machines; ATP driven; GroEL; Cryo-EM

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INTRODUCTION

Molecular chaperones are a set of proteins that maintain protein homeostasis in the cell and are essential for cell viability.[1–3] Chaperones are ubiquitous and constitutively expressed but most are also stress inducible. They interact with unfolded or partially folded proteins to stabilize non-native conformations, preventing aggregation and facilitating the correct folding or unfolding of proteins. They neither normally interact with native proteins nor do they form part of the final folded assembly. Some chaperones are nonspecific and interact with a wide variety of polypeptide chains, but others interact with specific targets. Many of them use ATP binding and/or hydrolysis as the energy source for their folding or unfolding activities. Moreover, as chaperones are predominantly involved in protein homeostasis they are implicated in conditions associated with its deregulation such as neurodegeneration and cancer.[4–6] Therefore understanding the functional mechanism of molecular chaperones is key to the comprehension and possible treatment of protein misfolding diseases.

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THE MAJOR ATP-DEPENDENT CYTOPLASMIC CHAPERONE FAMILIES

The following sections cover the general ATP-dependent chaperones of the cell and then focus on structure and function of the Hsp60 chaperonins.

The Hsp70 System

The Hsp70 chaperones form the most abundant chaperone family and are found in bacteria, eukaryotic cytosol, ER, mitochondria and chloroplasts, and in some archaea.[7–9] Like many other chaperones, they bind exposed hydrophobic regions and therefore can interact with a wide range of substrates. Their primary function in the cell is to bind to unfolded or partially unfolded proteins to prevent aggregation and to release them for folding. Hsp70's are also involved in cellular processes such as translation, translocation of proteins across membranes and apoptosis.[7,10–12] ATP binding and hydrolysis by Hsp70 are required for it to bind and release substrates[13–15] and are regulated by the obligatory co-chaperones Hsp40 (J-protein) and nucleotide exchange factors (e.g. GrpE, Hsp110). Hsp70 can deliver unfolded substrates to Hsp90 for refolding/activation.

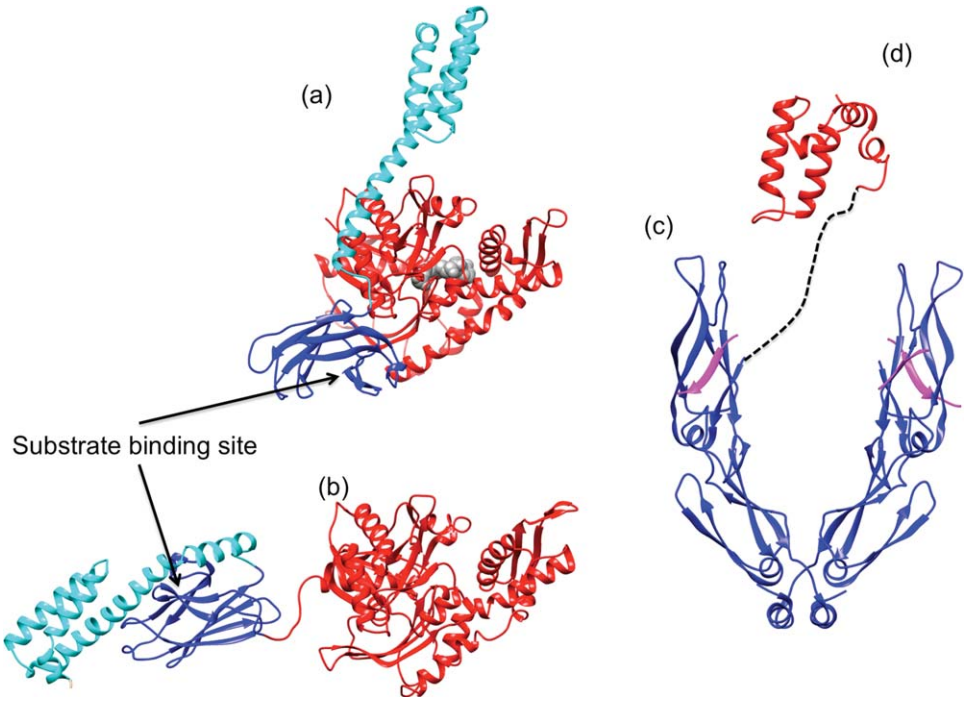

FIGURE 1 The Hsp70/Hsp40 chaperones. Structures of Hsp70 in the open, domain docked (a) and closed (b) conformations (PDB ID: 4B9Q and 2KHO). The nucleotide binding domains are shown in red (nucleotide is shown in gray), the substrate binding domains in blue and the C-terminal lid in cyan. (c) Structure of the C-terminal dimer of the peptide binding fragment of Hsp40 (blue) with three copies of the MEEVD peptide of Hsp70 bound to it (magenta) (PDB ID: 3AGY). Structure of the J-domain of Hsp40 (red) (PDB ID: 2O37). The black-dotted line shown in c and d indicates the connectivity of Hsp40 domains. All figures have been made with UCSF Chimera.[18]

Hsp70's are formed of a 44 kDa N-terminal ATPase domain and a 28 kDa substrate binding domain with a C-terminal lid subdomain[16,17] (Figure 1). The ATPase domain of Hsp70 has a similar fold to that of the functionally unrelated proteins actin and hexokinase.[19] The substrate-binding domain is a flat, brick shape with a channel that binds extended polypeptide chains, covered by the flexible lid subdomain. In the ATP bound conformation of Hsp70, the lid is more likely to be open, giving a low affinity for substrate.[20,21] ATP binding and subsequent closure of the nucleotide cleft creates a binding site on the ATPase domain for the interdomain linker. This linker docked conformation then recruits the substrate-binding domain to bind to the ATPase domain in a very open conformation favoring substrate binding.[20] Substrate binding stimulates the Hsp70 ATPase, releasing the substrate binding domain and allowing the lid subdomain to close over the substrate-binding site, locking the substrate in place.[22] A nucleotide exchange factor is required to release ADP and allow ATP into the nucleotide-binding site to re-open the lid and release substrate.[23,24] This resets Hsp70 ready for the next substrate and gives the released substrate the opportunity to fold correctly.

Hsp40 (J-domain proteins) are chaperones in their own right as they bind exposed hydrophobic residues and share common substrates with Hsp70.[25–27] Hsp40s contain a conserved 70 residue J-domain that interacts with the Hsp70 ATPase domain[28] (Figure 1). They also bind to the Hsp70 C-terminal EEVD motif via a C-terminal peptide-binding domain.[29,30] As a co-chaperone Hsp40 recruits substrate protein and facilitates its transfer to Hsp70 through interactions with both nucleotide and substrate binding domains of Hsp70. It also stimulates the ATPase activity of Hsp70, coordinating ATP hydrolysis and substrate binding. In addition to the general folding pathway, a variety of different Hsp40s can direct Hsp70 to specialized functions and sub-cellular regions.[7] For example the J-domain protein auxilin in combination with Hsp70 disassembles the clathrin coats of membrane vesicles after they have been internalized by clathrin mediated endocytosis.[31]

The Hsp90 System

Hsp90 was initially identified as a molecular chaperone that prevented aggregation of non-native proteins in an ATP

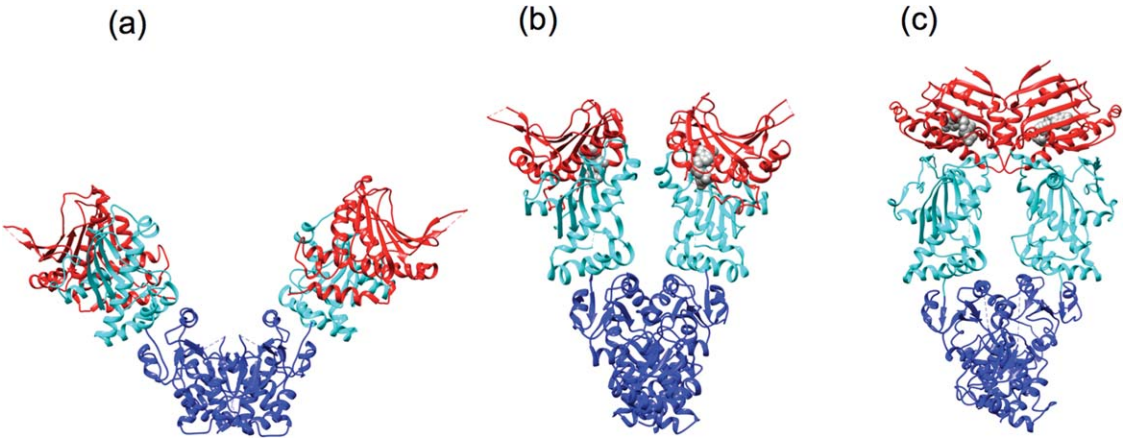

FIGURE 2 Conformations of Hsp90. The open (a), partially closed (b), and closed (c) conformations of the Hsp90 dimer (PDB ID: 2IOQ, 2O1U, and 2CG9). The N-terminal domains are shown in red, the middle domains in cyan and the C-terminal domains are shown in blue. Bound nucleotides are in gray.

dependent manner.[32,33] However, it appears to be more selective for substrates than other general chaperones[33] (http://www.picard.ch/downloads/downloads.htm). Hsp90 stabilizes proteins at later stages of folding. The majority of Hsp90 substrates can be classified into two types, the transcription factors, such as steroid hormone receptors and p53, and the signaling kinases, such as Cdk4. The cytosolic form of Hsp90 interacts with a number of co-chaperones that regulate its ATPase cycle and determine its substrate (client) proteins.[34–36] Another potential role for Hsp90 is that it could act as a buffer for genetic variation by rescuing mutated proteins with altered properties.[37] The functioning of such proteins could serve to increase the fitness of an organism during evolutionary change.[38]

Hsp90 has three conserved domains, the ATP binding N-terminal domain, the middle domain and the C-terminal dimerization domain which contains the MEEVD sequence that binds tetratricopeptide (TPR) containing co-chaperones[39,40] (Figure 2). Hsp90 functions as a dimer with a single intersubunit contact formed between the C-terminal domains in the absence of nucleotide.[41] ATP binding to the open structure induces a lid to close over the nucleotide binding site and the subsequent dimerization of the N-terminal domains forming a closed, twisted, compact conformation.[42] In this conformation, a flexible loop in the middle domain of each subunit makes contact with its N-terminal and substrate binding domains and initiates ATP hydrolysis. A potential route for the ATP induced conformational change in the middle domain has been suggested by molecular dynamics.[43] Once ATP is hydrolyzed the N-terminal domains dissociate to re-form the open conformation.[44] However, the nucleotide states are only weakly coupled to conformation, and the Hsp90 dimer exists in a dynamic equilibrium between open, closed and intermediate states.[45,46]

It is not clear if there is a specific client-binding site on Hsp90, since there is evidence for substrate protein interaction with all three domains. The kinase Cdk4 was shown by negative stain electron microscopy (EM) to interact with the middle domain of the Hsp90 dimer in an asymmetric fashion.[47] In other studies, the N- and C-terminal domains were also implicated in substrate protein binding.[48–51] Substrate proteins may also affect the conformation of Hsp90: a fragment of the staphylococcal nuclease has been shown to induce a partially closed conformation of Hsp90 and in combination with ATP it bound more tightly to the closed form and stimulated ATP hydrolysis.[52] Substrate proteins can also be passed from the Hsp70/40 complex to Hsp90 by the Hsp-organizing protein (HOP), which binds to the C-terminal MEEVD sequence of both Hsp90 and Hsp70 via separate TPR domains (TPR1 interacts with Hsp70 and TPR2A interacts with Hsp90).[53–55] HOP binding stabilizes an alternative open conformation of Hsp90 that inhibits ATP hydrolysis and facilitates client protein transfer from Hsp70.[55,56] Once the substrate is transferred, further Hsp90 co-chaperones such as peptidylprolyl isomerase (PPIases) are recruited to help client protein maturation. In contrast to HOP, p23 specifically binds to the N- and middle domains of the closed conformation of Hsp90.[42] Stabilizing the closed conformation slows ATP hydrolysis and thus facilitates the maturation of bound client proteins.[57] The co-chaperone Aha1 interacts with both the N- and middle domains of Hsp90 and its binding is proposed to promote dimerization of the N-domains, thus stimulating the ATPase.[58–60] Another key mode of regulation is through post-translational modification of Hsp90.[35]

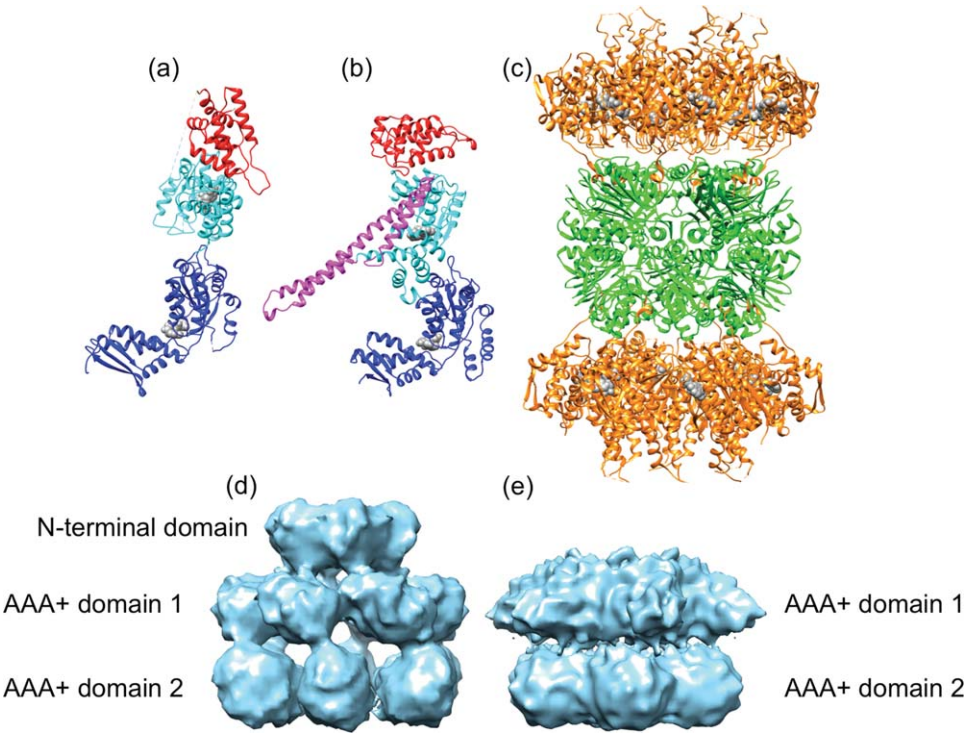

FIGURE 3 The Hsp100 chaperones. Monomer structures of ClpA (a), ClpB (b), and the HslUV chaperone protease complex (c) (PDB ID: 1KSF, 1QVR, and 1KYI). The nucleotide binding domains of ClpA and ClpB are shown in blue and cyan with the N-terminal domains shown in red, and bound nucleotides in gray. The coil-coiled insertion of ClpB, important for its disaggregation function, is shown in magenta. The chaperone HslU is shown in orange with the central ring protease HslV shown in green. The cryo-EM reconstructions of Hsp104-ATP and ClpB-ATP are shown in (d) and (e) (EM Databank ID: EMD-1600 and EMD-1244). The two AAA+ domains are labeled in both reconstructions but the N-terminal domain is only visible in the Hsp104 map.

The Hsp100 Unfoldases

The Hsp100/Clp proteins contain one or two conserved AAA+ (ATPases Associated with various cellular Activities) domains and act as unfoldases or disaggregases. A subset of the Hsp100 family of proteins (including ClpA, ClpX, and HslU) are coaxially stacked with a ring protease such as ClpP or HslV, and their functions are to unfold proteins and deliver them to the protease.[61,62] Another subset of Hsp100 proteins (ClpB, Hsp104, and Hsp101) function as disaggregases and have the unique ability, together with the Hsp70 system, to recover proteins from both amorphous and amyloid aggregates.[63,64] The main difference between the unfoldases and disaggregases is the presence of a coiled-coil insertion in the first AAA+ domain in the disaggregases. The Hsp100 proteins usually form hexamers which hydrolyze ATP in either a sequential/random or a concerted manner.[65–69] There is data to suggest that Hsp104 may have both positive and negative cooperativity between subunits during its ATPase cycle.[70]

Crystal structures have been determined of monomeric forms of several Hsp100 proteins, and of the hexamer forms of HslU, ClpX, and ClpC unfoldases[71–75] (Figure 3). Hexameric forms of various Hsp100's have been observed at intermediate resolutions by cryo-EM[66,67,72,76–79] (Figure 3). These structures, interpreted by docking of the available crystal structures, suggest a typical AAA+ packing arrangement for the unfoldases but an expanded conformation has been suggested for the Hsp104 disaggregase.[66,67] A typical AAA+ model has also been suggested for the disaggregases, but this model lacks density to account for the coiled-coil domain.[77]

The central channels of the Hsp100s are lined by tyrosine residues, located on mobile loops, which bind substrates non-specifically.[80] It is thought that rotations of the AAA+ domains provide the force to unfold the bound substrate and pull it through the channel.[81–83] The nature of these conformational changes is still unclear but kinetic results suggest that the subunits act in a sequential or random manner around the ring rather than a concerted one as in GroEL.[74,84] Disaggregation and unfolding functions are coupled and regulated via an interaction between the Hsp70 nucleotide-binding domain and the coiled-coil insertion (found in the first AAA+

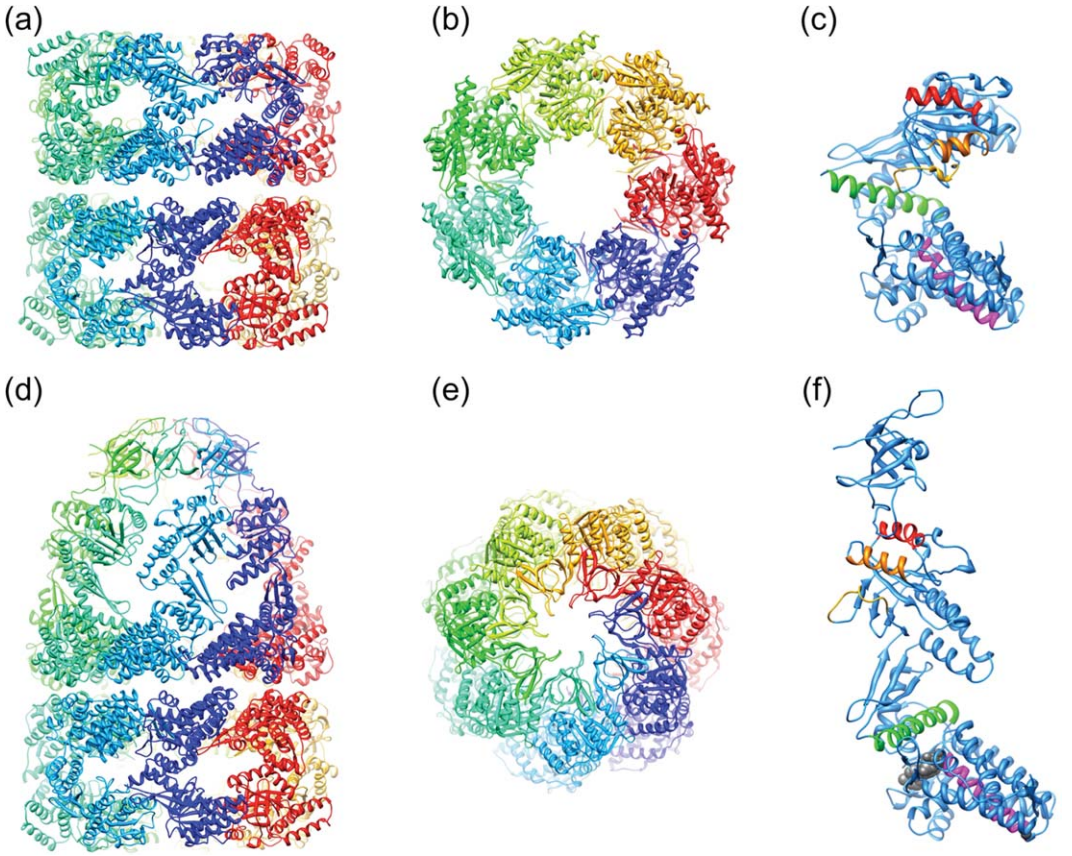

FIGURE 4 Structures of apo-GroEL and GroEL–GroES–ADP.AlF_3. Side (a,d) and top views (b,e) of unliganded GroEL and GroEL–GroES–ADP.AlF_3 complexes (PDB ID: 1OEL and 1SVT). Asymmetric units are shown in rainbow colors. Single subunits, viewed from inside the folding chamber, with helices H (red), I (orange), M (green), and D (magenta) for GroEL (c) and GroEL–GroES–ADP.AlF_3 (f). Nucleotide is in gray. Helix D has been suggested to transmit the nucleotide binding state of one ring to the opposite ring. Helices H and I bind the GroES mobile loop and unfolded substrate proteins. Helix M contains D398, which is important for ATP hydrolysis.

domain).[83,85,86] However, the precise location of the coiled-coil has been difficult to establish, since most of the hexamer structures so far determined contain little or no density to locate it. Recent biochemical and structural data suggest that it is docked on the outside surface of the AAA+ ring.[86,87]

GROEL–GROES (GROUP 1 CHAPERONINS)

The ATPase Cycle of GroEL

The function of the GroE chaperonins is to assist the folding of nascent polypeptides and the refolding of stress-denatured proteins.[1,2] It is arranged as two back-to-back rings, with each ring containing seven subunits and each subunit comprised of three distinct domains (Figure 4). The equatorial domains form the interface between the two GroEL rings and contain the sites of nucleotide binding and inter-ring communication. The two GroEL rings are staggered such that each equatorial domain interacts with two equatorial domains in the opposite ring. The apical domains line distal cavities containing the hydrophobic substrate and GroES binding sites (helices H, I and the underlying segment). Between the two major domains, is the intermediate domain that contains a catalytic residue important for ATP hydrolysis (located on helix M). GroEL is understood in atomic detail for two forms of the complex. The first form is the substrate binding, unliganded apo- or T-state in which both rings occupy the same conformation[88,89] (Figures 4a–4c). The second crystallographic conformation is the folding-active form of GroEL in which seven nucleotides and the heptameric co-chaperone GroES are bound to one of the GroEL rings[90,91] (Figures 4d–4f). In the GroES-bound ring (cis ring) the intermediate and apical domains exhibit large conformational changes relative to the apo state. The intermediate domain is rotated downward so that the M helix closes

over the nucleotide-binding site. The apical domain undergoes a large rotation and elevation which allow it to bind the mobile loop of GroES between helices H and I and to move the substrate binding site into the intersubunit interface, changing the cavity lining from hydrophobic to polar. These conformational changes form the Anfinsen cage, creating a protected environment favoring protein folding. The unliganded (trans) ring has a similar conformation to that of the apo ring.

ATP-bound conformations of the open ring have so far resisted crystallographic analysis. It is known that there is positive cooperativity within a GroEL ring favoring concerted binding of ATP to all seven subunits and negative cooperativity between rings.[92] Crystal structures of GroEL–ATP have been observed but they occupied the same conformation as the apo structures,[93] which is inconsistent with biochemical and biophysical data.[94–96] Cryo-EM has been the method of choice for determining GroEL–ATP conformations, with an earlier study showing that ATP binding causes rotations in all three domains of GroEL.[97] A small inward tilt of the equatorial domain lengthens the inter-ring contact at Ala109-Ala109, the likely route for transmission of negative cooperativity between the two GroEL rings. Ala109 is located at the bottom of helix D which is linked to the bound nucleotide, through Asp87, at its top end. The intermediate domain partly closes over the ATP binding pocket, bringing Asp398 in the M helix closer to the position that coordinates a water molecule in catalysis of ATP hydrolysis. The observed apical domain movements lead to the partial rotation of the substrate binding surface into the intersubunit interface and at the time was thought to be the trigger for ATP-induced substrate removal from GroEL.

Improvements in cryo-EM data collection and processing methods subsequently enabled a more detailed analysis of GroEL–ATP intermediate states[98] (Figure 5). This study revealed six GroEL–ATP conformations, three with ATP bound in one ring and three with ATP bound in both rings. The major domain movements are described in order of progression from apo-GroEL to the conformation closest to the GroES-bound structure. In the structure closest to the apo state, the apical and intermediate domain tilt en bloc, maintaining a continuous substrate binding surface on the inner face of the GroEL ring. This movement breaks the intersubunit salt bridges formed between Glu255-Lys207 and Arg197-Glu386 and replaces them with Glu255-Lys245 and Glu386-Lys80. In subsequent conformations, the apical domains elevate, eventually breaking the intra subunit salt bridge Asp83-Lys327, a contact important in GroES binding and ATP turnover.[99] The next big step is the separation and elevation of the apical domains so that the interapical domain salt bridges are broken and there is no longer a continuous substrate-binding surface. An intriguing feature of this open conformation is that the apical domains are positioned at the correct spacing to bind the mobile loops of the GroES heptamer but still present an essential part of the substrate binding site (helix I and the underlying segment—see later sections) to the cavity lining. The final change, only seen after GroES binding, is a 100° rotation of the apical domains that removes the substrate binding sites from the cavity wall and replaces them with a hydrophilic, net negatively charged surface. This final conformational change ejects bound substrate from GroEL, while simultaneously trapping it in the enclosed chamber for folding in isolation.

The multiple conformations of GroEL–ATP are compatible with observations from other studies. Kinetic observations on GroEL–ATP reveal at least three kinetic phases, consistent with the presence of multiple conformations seen by EM.[94–96] A fluorescent reporter located in the apical domain suggests that this domain moves in all three phases.[100] Second, maintenance of the substrate-binding surface in the ATP conformations is in agreement with interaction data.[101,102] Third, the open conformation has the potential to bind substrate and GroES at the same time.[103–105]

Formation of the GroEL–GroES–ATP complex gives the substrate ~10 s before ATP hydrolysis to reach its folded state.[106] Once the ATP is hydrolyzed the GroEL–GroES complex is primed for release. Upon GroES release, substrates leave the complex, and those that fail to fold can enter another round of GroEL–GroES interaction. A structural signal for priming GroES release has been observed in a cryo-EM study that showed a difference in the conformations of the trans rings between GroEL–GroES complexes formed with ATP or ADP in the cis ring. Only in the GroEL–GroES–ADP complex, the interequatorial domain beta sheet contact is broken[107] (Figure 6). Restoration of this contact by ATP binding to the trans ring would result in GroES ejection from the cis ring, releasing the contents of the folding chamber. At this step, a second GroES can bind before the first is released, resulting in transient complexes with GroES on both rings.[107–111]

GroEL–Substrate Interactions

GroEL binding prevents aggregation of many proteins but it is not able to recover proteins that have already aggregated.[2] The large number of proteins which GroEL binds in vivo, estimated to be around 10% of total *E. coli* protein, suggests that binding is nonspecific. The binding site is hydrophobic in character,[112] and contains essential hydrophobic residues that line the cavity-facing surface of the apical domain.[88,113] These residues are located on helices H and I and on the underlying extended segment of the apical domain. If one residue in this binding site is changed from hydrophobic to hydrophilic in character

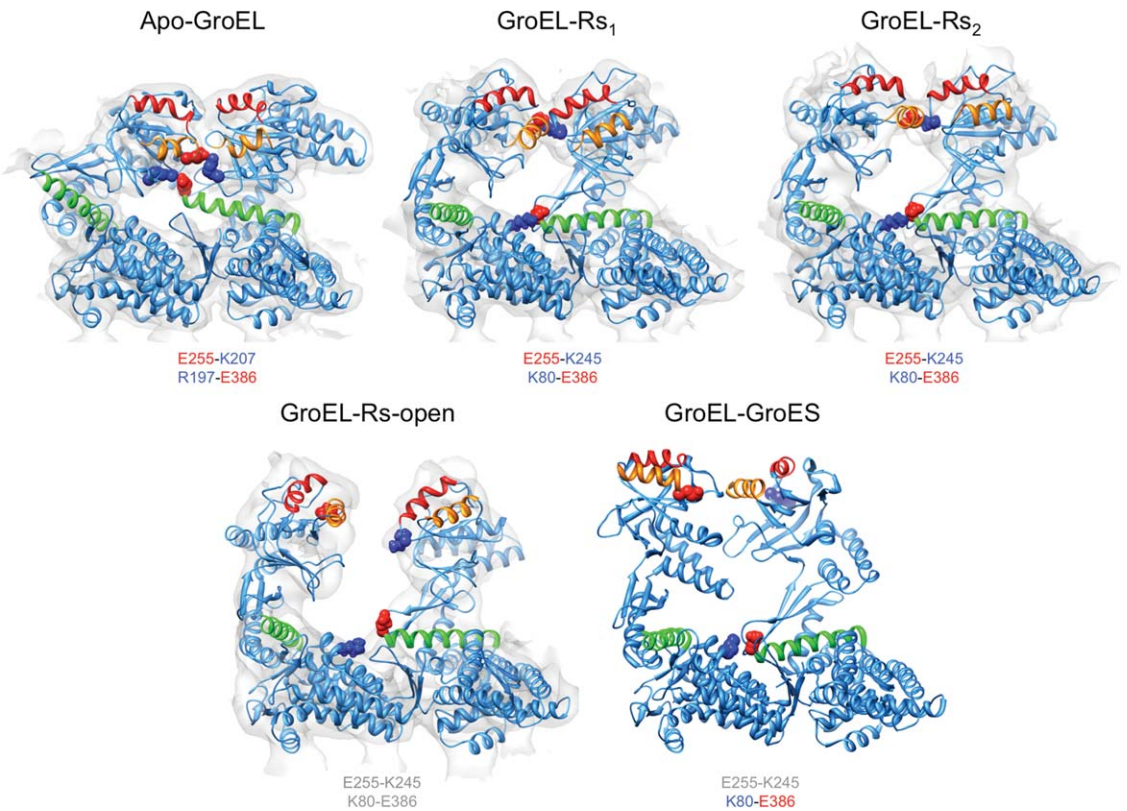

FIGURE 5 ATP induced conformational changes in GroEL. Two subunits, viewed from inside the folding chamber, of apo-GroEL, GroEL–Rs1, GroEL–Rs2, GroEL–Rs-open, and GroEL–GroES–ADP.AlF_3 complexes (EM Databank and PDB ID: EMD-1997/1OEL, EMD-1998/4AAQ, EMD-1999/4AAR, EMD-2000/4AAS, and 1SVT. Helices H (red), I (orange), and M (green) are highlighted as well as the residues involved in the intersubunit salt bridges (red and blue space fill).

(giving seven residues per ring) substrate binding is abolished in vitro and this GroEL mutant is not able to rescue GroEL knock out *E. coli* strains. However the binding surface of GroEL is not restricted to this area. Crosslinking experiments revealed that the bound substrate can interact with the whole inner face of a GroEL subunit.[114] Just the binding and release of substrates from the apical domains, without any encapsulation, is sufficient to fold some proteins[115] but stringent substrates such as Rubisco require encapsulation by GroES to reach their fully folded state.

The number and arrangement of binding sites in a GroEL ring are also important, suggesting that multiple binding sites

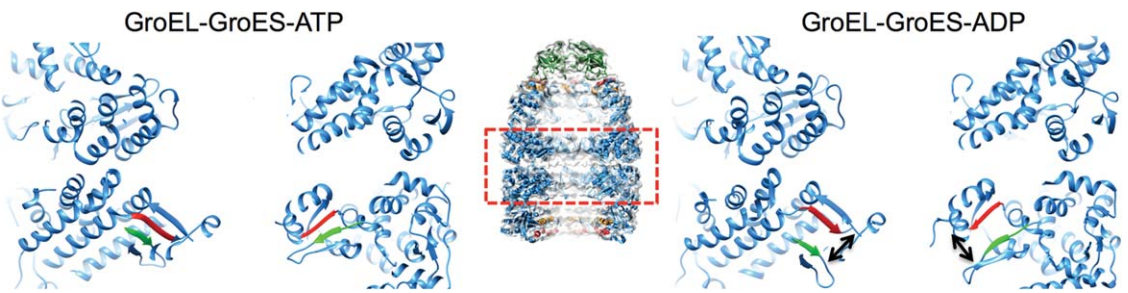

FIGURE 6 Structural basis for the priming of GroEL for GroES release. Slice through the centre of GroEL–GroES–ATP and ADP complexes with the intersubunit β-sheet shown in green and red for the equatorial domains in the trans ring (ring opposite GroES) (EM Databank and PDB ID: EMD-1180/2C7C and EMD-1181/2C7D). Black double-headed arrows at the equatorial domain of GroEL–GroES–ADP highlight the intersubunit β-sheet expansion.

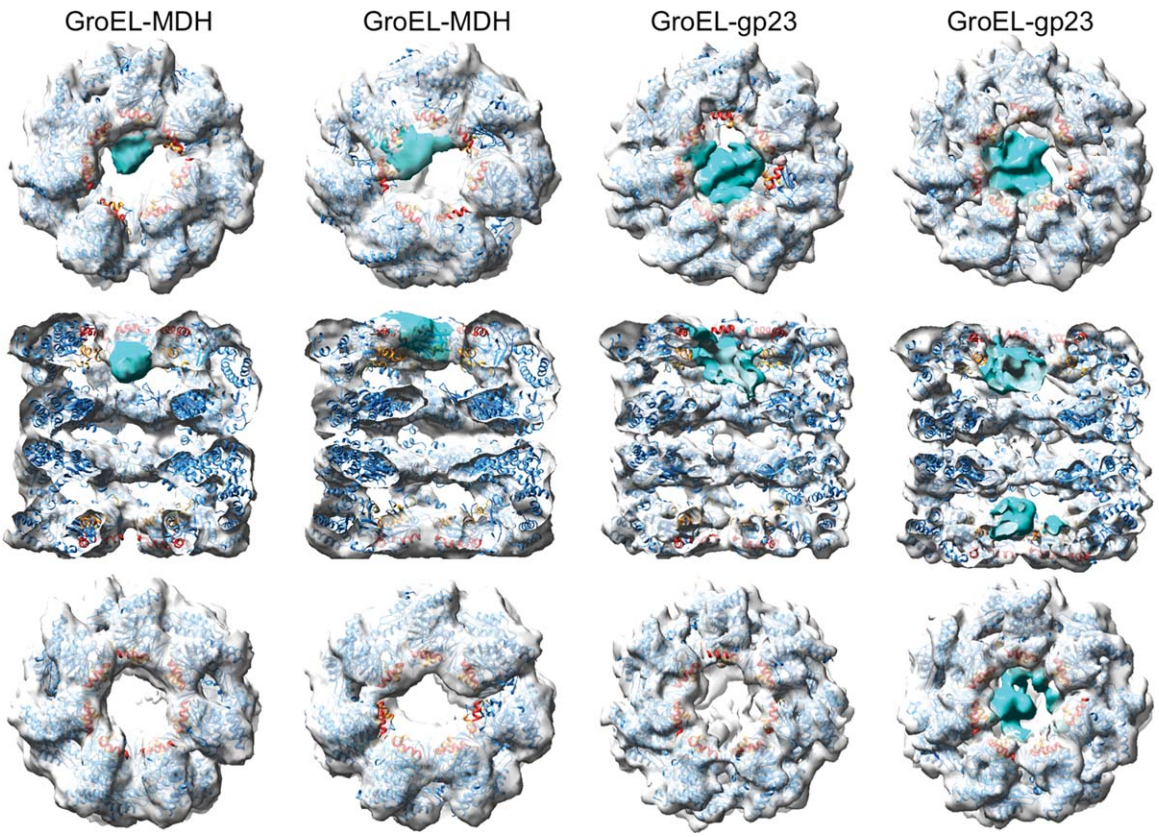

FIGURE 7 Structures of GroEL complexes with non-native MDH and gp23. Cryo-EM maps and fits for GroEL–MDH (left-hand two columns) and GroEL–gp23 (right-hand two columns) shown from the top, side, and bottom (EM Databank ID for GroEL–gp23 complexes: EMD-1544 and EMD-1545). GroEL density is shown as a white transparent surface with the substrate density shown as cyan. Helices H and I are shown as red and orange with the rest of the coordinates shown in blue. Two structures of GroEL–MDH are shown, from an ensemble of five structures that were determined by classification of a heterogeneous data set. The same approach was used for the GroEL–gp23 complexes.

act together as a continuous hydrophobic binding surface. This was demonstrated by a study in which all seven subunits of a GroEL ring were covalently linked and expressed as a single polypeptide chain.[116] This allowed binding sites to be selectively inactivated. From this it was shown that stringent substrates require at least three contiguous binding sites for productive folding and that two noncontiguous sites are sufficient for less stringent substrates. In addition, disulphide crosslinking experiments showed direct interactions in which 3–4 GroEL subunits could be crosslinked to an unfolded Rubisco. Subsequently, cryo-EM reconstructions showed that substrates bind to multiple apical domains.[114,117,118]

A number of studies have shown that proteins stably bound to GroEL are unstructured and that binding of non-native proteins to GroEL can be associated with unfolding.[119–121] The difficult problem of probing the structure of GroEL complexes with non-native proteins has been approached by a range of techniques. X-ray crystallographic studies have revealed structures of extended or helical peptides bound in the groove formed by helices H and I via hydrophobic interactions.[122–124] This binding resembles that of the mobile loop of GroES. However, the apical domains must be able to bind substrate at other sites since GroES-like peptides and substrate derived helical peptides can bind simultaneously.[103] The additional binding site is probably located in the underlying segment of the apical domain, residues 199–209, which are essential for binding Rubisco and MDH.

Cryo-EM has also been used to probe the structure of non-native proteins bound to GroEL. Initial studies observed that the substrates were bound to helices H and I, with substrate density protruding from the GroEL ring.[117,125,126] In a more detailed cryo-EM study, denatured MDH was shown to occupy multiple positions on GroEL. Most complexes showed substrate density at helix I and the underlying segment in 3–4

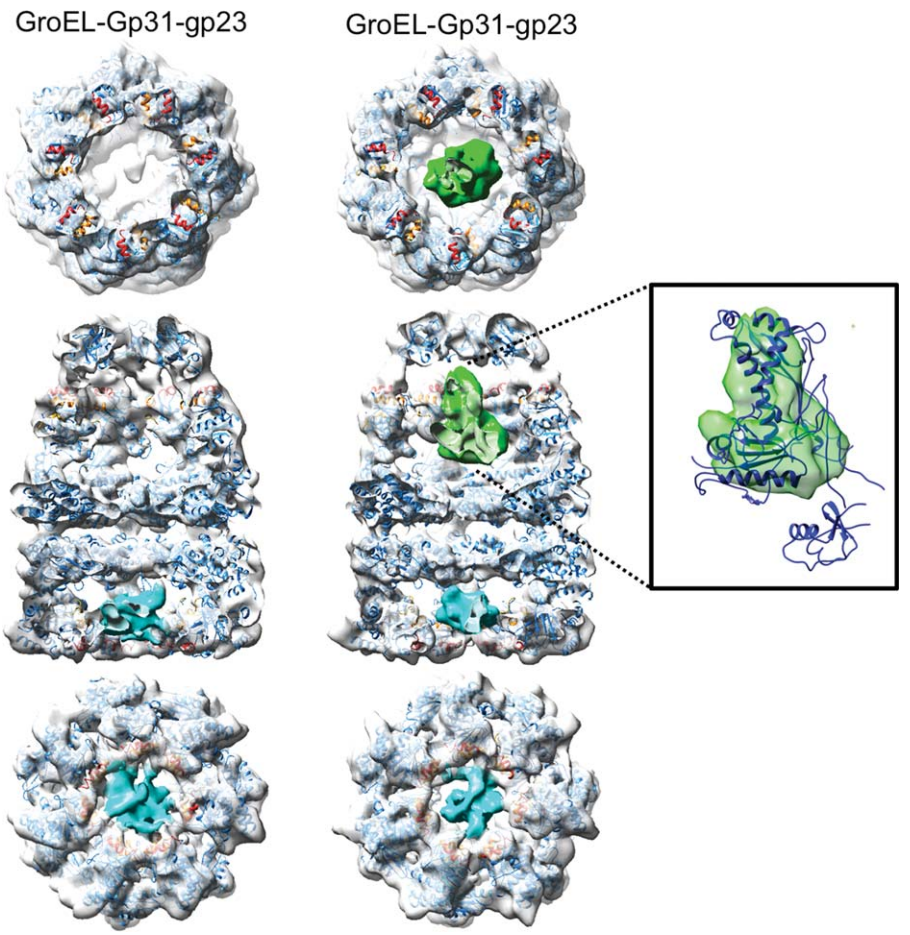

FIGURE 8 Structures of GroEL–gp31-ADP.ALF$_3$ complexes with folding gp23. Cryo-EM maps and fits of the GroEL–Gp31–ADP.ALF$_3$ complex with substrate protein gp23 bound in the open trans ring (left-hand column; EMD-1547) and in the folding chamber and open trans ring (right-hand column; EMD-1548). The GroEL–gp31 cryo-EM densities are shown as white transparent surfaces. gp23 densities in the open trans ring are shown in cyan and the gp23 in the folding chamber in green. Atomic coordinates are colored as in Figure 7. The inset on the right shows the gp23 density with the coordinates of the closely related capsid protein gp24 (blue) (PDB ID: 1YUE) placed in the density. The gp23 density in the trans ring occupies a similar position to that seen in the GroEL–gp23 complexes.

apical domains, but one had a bilobed appearance reminiscent of the folded form of MDH located closer to the entrance of the cavity[114] (Figure 7, left 2 panels).

There is an upper limit, around 60 kDa, to the size of substrate that can fit inside the folding chamber. The major capsid protein, gp23, of bacteriophage T4 is a 56 kDa stringent substrate of GroEL. The bacteriophage uses the host *E. coli* GroEL during infection but makes its own co-chaperonin, gp31, instead of GroES in the folding reaction of gp23 to make a slightly taller cavity.[127] GroEL–gp23 complexes had similar features to two of the GroEL–MDH complexes in that substrate density was mainly seen adjacent to helix I and the underlying segment deep within in the GroEL ring.[118] This study also confirmed mass-spectrometry observations showing GroEL complexes with gp23 bound to both rings[128,129] (Figure 7, right 2 panels).

The study with GroEL and gp23 provided the first view of a substrate encapsulated in the folding chamber. The folding active conformation was formed with GroEL and the phage GroES analog gp31 using the ATP analog ADP·AlF$_3$. In this complex, the cis ring contained distinct density for gp23 and docking of the related crystal structure gp24 clearly showed that the large domain of gp23 was close to its native shape (Figure 8). The structure also suggested that the 56 kDa gp23 exerts pressure on the folding chamber, which was expanded, with a gap between two of the apical domains.

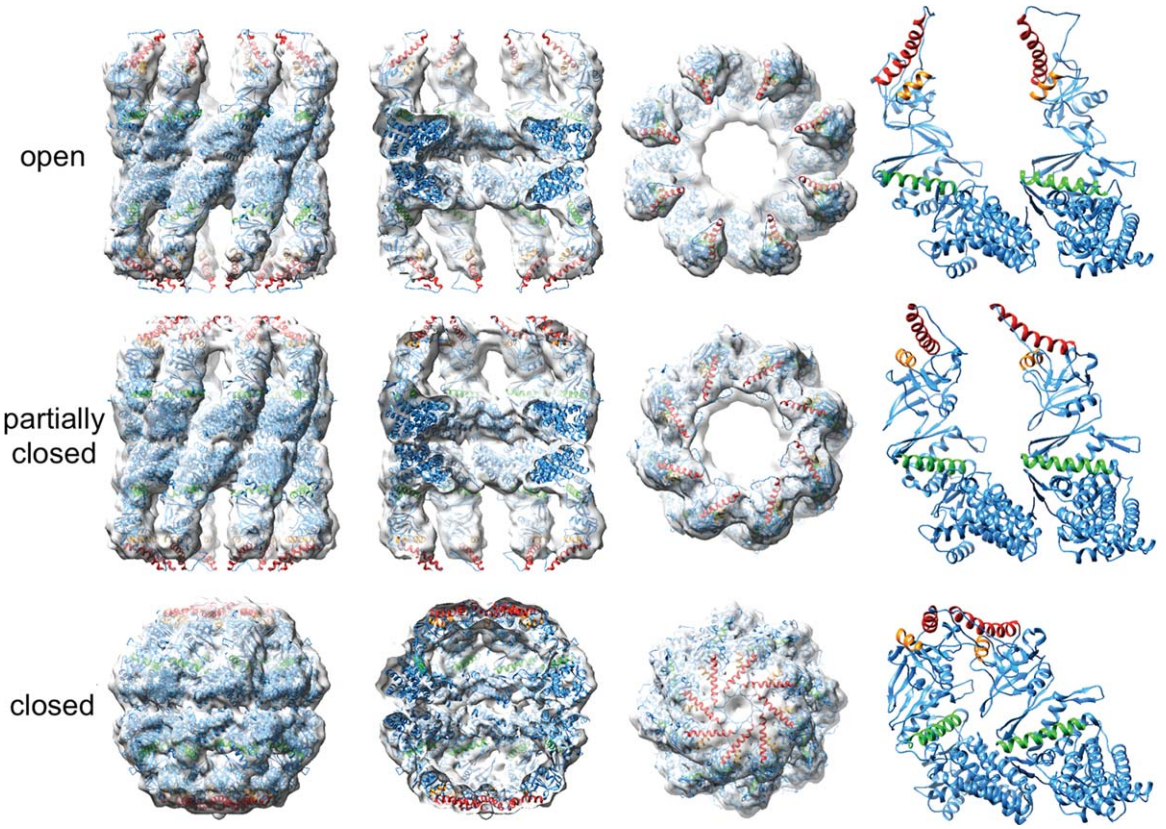

FIGURE 9 Multiple conformations of a Group 2 chaperonin. Cryo-EM maps of the Group 2 chaperonin from *Methanococcus maripaludis* in the open (top row), partially closed (middle row) and closed conformation shown in side view, cut away side view and top view (EM Databank and PDB ID: EMD-1396, EMD-1397, and EMD-1398/1A6D). The cryo-EM densities are shown as a white transparent surface with the Group 2 equivalents of helices H, I, and M from Group 1 shown in red, orange, and green, respectively. The final column shows two adjacent subunits, viewed from inside the folding chamber, for each of the conformations.

Folding Machinery

Structural, biochemical, and biophysical studies have shown how substrate proteins interact with GroEL and how ATP induces conformational changes that convert GroEL from substrate binding to substrate folding conformation. From mutational analysis and cryo-EM studies it is clear that substrates bind primarily to helix I and the underlying segment and that they bind to multiple apical domains simultaneously. Bound substrate would then be subjected to apical ring extension and expansion during ATP induced conformational changes, suggesting the mechanism for forced unfolding of the substrate by GroEL.[98,121,130,131] Notably, bound substrate presents a mechanical load on GroEL domain movement.[132] Substrates binding after ATP would probably preferentially bind to conformations with a continuous hydrophobic binding surface, and they would still be subjected to the extension and expansion of the apical domains. The 100° rotation of the apical domains from the open to the GroES-bound state would forcibly eject the substrate by removing its binding sites from the inside of the chamber and trap in it the GroES capped folding chamber.

GROUP 2 CHAPERONINS

GroEL has both eukaryotic and archaeal homologues (CCT/TRIC in eukaryotes and the thermosome in Archaea), the Group 2 chaperonins which are also essential for folding proteins. Members of this chaperonin subfamily have 8- or 9-fold symmetry, forming back-to-back rings in homo- or hetero-oligomeric complexes with the individual subunits retaining the same domain structure and a high degree of sequence identity/similarity to GroEL (thermosome alpha subunit has 46% identity to GroEL[133] and CCT has 17–20% identity and 60–63% similarity to GroEL[134]). The main difference is an extension inserted into the helix H equivalent in the apical domain that forms the lid of the folding chamber, removing the

requirement for a GroES like co-chaperone.[133,135] Group 2 chaperonins also have a different inter-ring interface with an in register, one-to-one arrangement rather than the one-to-two arrangement of opposite rings seen in GroEL. The intersubunit interface in CCT is more complicated because each ring contains eight distinct subunits and the interface is formed only between specific subunits.[136,137] It is unclear whether CCT, which is not a heat shock protein, uses a hydrophobic substrate-binding surface such as GroEL or makes more specific contacts with its substrates (there is evidence for both). It is clear that some CCT substrates, notably actin and tubulin, bind to specific CCT subunits. Archaeal thermosomes are thought to be general chaperones that use a similar hydrophobic binding surface to that of GroEL. These complexes are generally formed of one or two different subunit types and can fold some model GroEL substrates.[138]

Structural studies of the Group 2 chaperonins in different nucleotide bound states reveal open, substrate binding and closed, substrate folding conformations similar to GroEL[139–145] (Figure 9). The most open conformation resembles the open form of GroEL–ATP (R-open states), with very mobile and separated subunit apical domains.[139,140,144] This state has only been observed by cryo-EM, since the crystallographic open structure reported by Huo and coworkers in 2011 is similar to the partially closed conformation observed by cryo-EM.[139,140,142] Conversion from the open to the partially closed conformation involves a large clockwise twist of the apical domains, which brings the helical protrusions close enough to make an initial contact. In the fully closed conformation, the subunits tip inward toward the symmetry axis of the complex with the helical protrusions joining to form the lid of the folding chamber.

The Group 2 conformational changes have many similarities with those in GroEL, but order of tilting and twisting appears to be reversed. This can be seen by comparing movies of the conformational changes in the two subfamilies (Supporting Information Movies 1 and 2). From the open conformation the apical domains first twist clockwise by 30°, resembling the movement from GroEL–Rs-open to GroEL–GroES. From the partly to fully closed conformation, a 20° inward tilt of the whole subunit brings the apical domains together, a movement similar to the GroEL–Rs1 to GroEL–Rs-open transition but in the reverse direction. This downward tilt brings the catalytic Asp in the intermediate domain close to the ATP binding site and closes the folding chamber. The ring expansion/contraction of Group 2 chaperonins is facilitated by the 1:1 nature of their inter-ring interface, allowing the equatorial domains to move more freely than in GroEL. There is evidence to suggest that in CCT these movements occur through a random or sequential ATPase action instead of a concerted one like GroEL.[146] However, the archaeal Group 2 chaperonins have a concerted ATPase cycle.[147,148]

SUMMARY

ATP-driven chaperones play key roles in protein homeostasis, as they bind, unfold, refold or disaggregate non-native proteins. ATP binding and hydrolysis in the Hsp70 system regulate substrate binding and release. The Hsp70 ATP cycle is also regulated by a variety of co-chaperones. Hsp90 uses its ATPase cycle to induce multiple conformations that bind and stabilize or assist maturation of substrate proteins. Hsp90's ATPase cycle is also regulated by numerous co-chaperones. The Hsp100s use ATP to unfold, thread and disaggregate substrate proteins. In ATP-dependent proteolysis, the unfoldase is coupled to a protease which degrades the unfolded substrate proteins. The disaggregases in combination with the Hsp70 system use ATP-induced conformational changes to disaggregate and unfold substrate proteins. GroEL–GroES uses ATP binding to induce conformational changes to convert from a substrate binding to a substrate folding complex. It may also use the ATP-induced conformational changes to forcibly unfold bound substrates. GroES binding ejects the substrate from the binding surface, giving it a chance to fold in isolation during the slow ATP hydrolysis step. The archaeal Group 2 chaperonins appear to operate via similar ATP-induced domain rotations to those in GroEL but the conformational changes are not in the same order. The eukaryotic cytosol Group 2 chaperonin resembles the archaeal system, but its ATPase cycle and action appear to be more complex and substrate specific.

REFERENCES

1. Hartl, F. U.; Hayer-Hartl, M. Science 2002, 295, 1852–1858.
2. Fenton, W. A.; Horwich, A. L. Q Rev Biophys 2003, 36, 229–256.
3. Bukau, B.; Weissman, J.; Horwich, A. L. Cell 2006, 125, 443–451.
4. Hartl, F. U.; Hayer-Hartl, M. Nat Struct Mol Biol 2009, 16, 574–581.
5. Hartl, F. U.; Bracher, A.; Hayer-Hartl, M. Nature 2011, 475, 324–332.
6. Neckers, L.; Mimnaugh, E.; Schulte, T. W. Drug Resist Updat 1999, 2, 165–172.
7. Kampinga, H. H.; Craig, E. A. Nat Rev Mol Cell Biol 2010, 11, 579–592.
8. Sharma, D.; Masison, D. C. Protein Pept Lett 2009, 16, 571–581.
9. Gribaldo, S.; Lumia, V.; Creti, R.; Conway de Macario, E.; Sanangelantoni, A.; Cammarano, P. J Bacteriol 1999, 181, 434–443.
10. De Los Rios, P.; Ben-Zvi, A.; Slutsky, O.; Azem, A.; Goloubinoff, P. Proc Natl Acad Sci USA 2006, 103, 6166–6177.
11. Floer, M.; Bryant, G. O.; Ptashne, M. Proc Natl Acad Sci USA 2008, 105, 2975–2980.
12. Weiss, Y. G.; Maloyan, A.; Tazelaar, J.; Raj, N.; Deutschman, C. S. J Clin Invest 2002, 110, 801–806.

13. Szabo, A.; Langer, T.; Schröder, H.; Flanagan, J.; Bukau, B.; Hartl, F. U. Proc Natl Acad Sci USA 1994, 91, 10345–10349.
14. Freeman, B. C.; Mvers, M. P.; Schumacher, R.; Morimoto, R. I. EMBO J 1995, 14, 2281–2292.
15. Freeman, B. C.; Morimoto, R. I. EMBO J 1996, 15, 2969–2979.
16. Flaherty, K. M.; DeLuca-Flaherty, C.; McKay, D. B. Nature 1990, 346, 623–628.
17. Zhu, X.; Zhao, X.; Burkholder, W. F.; Gragerov, A.; Ogata, C. M.; Gottesman, M. E.; Hendrickson, W. A. Science 1996, 272, 1606–1614.
18. Goddard, T. D.; Huang, C. C.; Ferrin, T. E. J Struct Biol 2007, 157, 281–287.
19. Bork, P.; Sander, C.; Valencia, A. Proc Natl Acad Sci USA 1992, 89, 7290–7294.
20. Kityk, R.; Kopp, J.; Sinning, I.; Mayer, M. P. Mol Cell 2012, 48, 863–874.
21. Zhuravleva, A.; Clerico, E. M.; Gierasch, L. M. Cell 2012, 151, 1296–1307.
22. Bertelsen, E. B.; Chang, L.; Gestwicki, J. E.; Zuiderweg, E. R. Proc Natl Acad Sci USA 2009, 106, 8471–8476.
23. Liberek, K.; Marszalek, J.; Ang, D.; Georgopoulos, C.; Zylicz, M. Proc Natl Acad Sci USA 1991, 88, 2874–2878.
24. Harrison, C. J.; Hayer-Hartl, M.; Di Liberto, M.; Hartl, F. U.; Kuriyan, J. Science 1997, 276, 431–435.
25. Szabo, A.; Korszun, R.; Hartl, F. U.; Flanagan, J. EMBO J 1996, 15, 408–417.
26. Goffin, L.; Georgopoulos, C. Mol Microbiol 1998, 30, 329–340.
27. Johnson, J. L.; Craig, E. A. J Cell Biol 2001, 152, 851–856.
28. Ahmad, A.; Bhattacharya, A.; McDonald, R. A.; Cordes, M.; Ellington, B.; Bertelsen, E. B.; Zuiderweg, E. R. Proc Natl Acad Sci USA 2011, 108, 18966–18971.
29. Li, J.; Qian, X.; Sha, B. Protein Pept Lett 2009, 16, 606–612.
30. Suzuki, H.; Noguchi, S.; Arakawa, H.; Tokida, T.; Hashimoto, M.; Satow, Y. Biochemistry 2010, 49, 8577–8584.
31. Rothnie, A.; Clarke, A. R.; Kuzmic, P.; Cameron, A.; Smith, C. J. Proc Natl Acad Sci USA 2011, 108, 6927–6932.
32. Wiech, H.; Buchner, J.; Zimmermann, R.; Jakob, U. Nature 1992, 358, 169–170.
33. Picard, D. Cell Mol Life Sci 2002, 59, 1640–1648.
34. Zuehlke, A.; Johnson, J. L. Biopolymers 2010, 93, 211–217.
35. Li, J.; Soroka, J.; Buchner, J. Biochim Biophys Acta 2012, 1823, 624–635.
36. Johnson, J. L. Biochim Biophys Acta 2012, 1923, 607–613.
37. Taipale, M.; Jarosz, D. F.; Lindquist, S. Nat Rev Mol Cell Biol 2010, 11, 515–528.
38. Jarosz, D. F.; Lindquist, S. Science 2010, 330,1820–1824.
39. Prodromou, C.; Roe, S. M.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. Cell 1997, 90, 65–75.
40. Young, J. C.; Obermann, W. M.; Hartl, F. U. J Biol Chem 1998, 273, 18007–18010.
41. Shiau, A. K.; Harris, S. F.; Southworth, D. R.; Agard, D. A. Cell 2006, 127, 329–340.
42. Ali, M. M.; Roe, S. M.; Vaughan, C. K.; Meyer, P.; Panaretou, B.; Piper, P. W.; Prodromou, C.; Pearl, L. H. Nature 2006, 440, 1013–1017.
43. Seifert, C.; Gräter, F. Biophys J 2012, 103, 2195–2202.
44. Dollins, D. E.; Warren, J. J.; Immormino, R. M.; Gewirth, D. T. Mol Cell 2007, 28, 41–56.
45. Southworth, D. R.; Agard, D. A. Mol Cell 2008, 32, 631–640.
46. Mickler, M.; Hessling, M.; Ratzke, C.; Buchner, J.; Hugel, T. Nat Struct Mol Biol 2009, 16, 281–286.
47. Vaughan, C. K.; Gohlke, U.; Sobott, F.; Good, V. M.; Ali, M. M.; Prodromou, C.; Robinson, C. V.; Saibil, H. R.; Pearl, L. H. Mol Cell 2006, 23, 697–707.
48. Young, J. C.; Schneider, C.; Hartl, F. U. FEBS Lett 1997, 418, 139–143.
49. Scheibel, T.; Weikl, T.; Buchner, J. Proc Natl Acad Sci USA 1998, 95, 1495–1499.
50. Fang, L.; Ricketson, D.; Getubig, L.; Darimont, B. Proc Natl Acad Sci USA 2006, 103, 18487–18492.
51. Park, S. J.; Kostic, M.; Dyson, H. J. J Mol Biol 2011, 411, 158–173.
52. Street, T. O.; Lavery, L. A.; Agard, D. A. Mol Cell 2011, 42, 96–105.
53. Chen, S.; Smith, D. F. J Biol Chem 1998, 273, 35194–35200.
54. Kajander, T.; Sachs, J. N.; Goldman, A.; Regan, L. J Biol Chem 2009, 284, 25364–25374.
55. Southworth, D. R.; Agard, D. A. Mol Cell 2011, 42, 771–781.
56. Prodromou, C.; Siligardi, G.; O'Brien, R.; Woolfson, D. N.; Regan, L.; Panaretou, B.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. EMBO J 1999, 18, 754–762.
57. McLaughlin, S. H.; Sobott, F.; Yao, Z. P.; Zhang, W.; Nielsen, P. R.; Grossmann, J. G.; Laue, E. D.; Robinson, C. V.; Jackson, S. E. J Mol Biol 2006, 356, 746–758.
58. Lotz, G. P.; Lin, H.; Harst, A.; Obermann, W. M. J Biol Chem 2003, 278, 17228–17235.
59. Meyer, P.; Prodromou, C.; Liao, C.; Hu, B.; Mark Roe, S.; Vaughan, C. K.; Vlasic, I.; Panaretou, B.; Piper, P. W.; Pearl, L. H. EMBO J 2004, 23, 511–519.
60. Retzlaff, M.; Hagn, F.; Mitschke, L.; Hessling, M.; Gugel, F.; Kessler, H.; Richter, K.; Buchner, J. Mol Cell 2010, 37, 344–354.
61. Butler, S. M.; Festa, R. A.; Pearce, M. J.; Darwin, K. H. Mol Microbiol 2006, 60, 553–562.
62. Kress, W.; Maglica, Z.; Weber-Ban, E. Res Microbiol 2009, 160, 618–628.
63. Winkler, J.; Tyedmers, J.; Bukau, B.; Mogk, A. J Struct Biol 2012, 179, 152–160.
64. Desantis, M. E.; Shorter, J. Biochim Biophys Acta 2012, 1823, 29–39.
65. Martin, A.; Baker, T. A.; Sauer, R. T. Nature 2005, 437, 1115–1120.
66. Wendler, P.; Shorter, J.; Plisson, C.; Cashikar, A. G.; Lindquist, S.; Saibil, H. R. Cell 2007, 131, 1366–1377.
67. Wendler, P.; Shorter, J.; Snead, D.; Plisson, C.; Clare, D. K.; Lindquist, S.; Saibil, H. R. Mol Cell 2009, 34, 81–92.
68. Gai, D.; Zhao, R.; Li, D.; Finkielstein, C. V.; Chen, X. S. Cell 2004, 119, 47–60.
69. Beuron, F.; Dreveny, I.; Yuan, X.; Pye, V. E.; McKeown, C.; Briggs, L. C.; Cliff, M. J.; Kaneko, Y.; Wallis, R.; Isaacson, R. L.; Ladbury, J. E.; Matthews, S. J.; Kondo, H.; Zhang, X.; Freemont, P. S. EMBO J 2006, 25, 1967–1976.
70. Franzmann, T. M.; Czekalla, A.; Walter, S. G. J Biol Chem 2011, 286, 17992–8001.
71. Guo, F.; Maurizi, M. R.; Esser, L.; Xia, D. J Biol Chem 2002, 277, 46743–46752.

72. Lee, S.; Sowa, M. E.; Watanabe, Y. H.; Sigler, P. B.; Chiu, W.; Yoshida, M.; Tsai, F. T. Cell 2003, 115, 229–240.
73. Kwon, A. R.; Kessler, B. M.; Overkleeft, H. S.; McKay D. B. J Mol Biol 2003, 330, 185–195.
74. Glynn, S. E.; Martin, A.; Nager, A. R.; Baker, T. A.; Sauer, R. T. Cell 2009, 139, 744–756.
75. Wang, F.; Mei, Z.; Qi, Y.; Yan, C.; Hu, Q.; Wang, J.; Shi, Y. Nature 2011, 471, 331–335.
76. Lee, S.; Choi, J. M.; Tsai, F. T. Mol Cell 2007, 25, 261–271.
77. Lee, S.; Sielaff, B.; Lee, J.; Tsai, F. T. Proc Natl Acad Sci USA 2010, 107, 8135–8140.
78. Beuron, F.; Maurizi, M. R.; Belnap, D. M.; Kocsis, E.; Booy, F. P.; Kessel, M.; Steven, A. C. J Struct Biol 1998, 123, 248–259.
79. Effantin, G.; Ishikawa, T.; De Donatis, G. M.; Maurizi, M. R.; Steven, A. C. Structure 2010, 18, 553–562.
80. Hinnerwisch, J.; Fenton, W. A.; Furtak, K. J.; Farr, G. W.; Horwich, A. L. Cell 2005, 121, 1029–1041.
81. Weber-Ban, E. U.; Reid, B. G.; Miranker, A. D.; Horwich, A. L. Nature 1999, 401, 90–93.
82. Singh, S. K.; Grimaud, R.; Hoskins, J. R.; Wickner, S.; Maurizi, M. R. Proc Natl Acad Sci USA 2000, 97, 8898–8903.
83. Weibezahn, J.; Tessarz, P.; Schlieker, C.; Zahn, R.; Maglica, Z.; Lee, S.; Zentgraf, H.; Weber-Ban, E. U.; Dougan, D. A.; Tsai, F. T.; Mogk, A.; Bukau, B. Cell 2004, 119, 653–665.
84. Maillard, R. A.; Chistol, G.; Sen, M.; Righini, M.; Tan, J.; Kaiser, C. M.; Hodges, C.; Martin, A.; Bustamante, C. Cell 2011, 145, 459–469.
85. Seyffer, F.; Kummer, E.; Oguchi, Y.; Winkler, J.; Kumar, M.; Zahn, R.; Sourjik, V.; Bukau, B.; Mogk, A. Nat Struct Mol Biol 2012, 19, 1347–1355.
86. Rosenzweig, R.; Moradi, S.; Zarrine-Afsar, A.; Glover, J. R.; Kay, L. E. Science 2013, 339, 1080–1083.
87. Oguchi, Y.; Kummer, E.; Seyffer, F.; Berynskyy, M.; Anstett, B.; Zahn, R.; Wade, R. C.; Mogk, A.; Bukau, B. Nat Struct Mol Biol 2012, 19, 1338–1346.
88. Braig, K.; Otwinowski, Z.; Hegde, R.; Boisvert, D. C.; Joachimiak, A.; Horwich, A. L.; Sigler, P. B. Nature 1994, 371, 578–586.
89. Braig, K.; Adams, P. D. Brünger, A. T. Nat Struct Biol 1995, 2, 1083–1094.
90. Xu, Z.; Horwich, A. L.; Sigler, P. B. Nature 1997, 388, 741–750.
91. Chaudhry, C.; Farr, G. W.; Todd, M. J.; Rye, H. S.; Brunger, A. T.; Adams, P. D.; Horwich, A. L.; Sigler, P. B. EMBO J 2003, 22, 4877–4887.
92. Yifrach, O.; Horovitz, A. Biochemistry 1995, 34, 5303–5308.
93. Wang, J.; Boisvert, D. C. J Mol Biol 2003, 327, 843–855.
94. Yifrach, O.; Horovitz, A. Biochemistry 1998, 37, 7083–7088.
95. Cliff, M. J.; Kad, N. M.; Hay, N.; Lund, P. A.; Webb, M. R.; Burston, S. G.; Clarke, A. R. J Mol Biol 1999, 293, 667–684.
96. Kovács, E.; Sun, Z.; Liu, H.; Scott, D. J.; Karsisiotis, A. I.; Clarke, A. R.; Burston, S. G.; Lund, P. A. J Mol Biol 2010, 396, 1271–1283.
97. Ranson, N. A.; Farr, G. W.; Roseman, A. M.; Gowen, B.; Fenton, W. A.; Horwich, A. L.; Saibil, H. R. Cell 2001, 107, 869–879.
98. Clare, D. K.; Vasishtan, D.; Stagg, S.; Quispe, J.; Farr, G. W.; Topf, M.; Horwich, A. L.; Saibil, H. R. Cell 2012, 149, 113–123.
99. Murai, N.; Makino, Y.; Yoshida, M. J Biol Chem 1996, 271, 28229–28234.
100. Taniguchi, M.; Yoshimi, T.; Hongo, K.; Mizobata, T.; Kawata, Y. J Biol Chem 2004, 279, 16368–16376.
101. Tyagi, N. K.; Fenton, W. A.; Horwich, A. L. Proc Natl Acad Sci USA 2009, 106, 20264–20269.
102. Koike-Takeshita, A.; Yoshida, M.; Taguchi, H. J Biol Chem 2008, 283, 23774–23781.
103. Ashcroft, A. E.; Brinker, A.; Coyle, J. E.; Weber, F.; Kaiser, M.; Moroder, L.; Parsons, M. R.; Jager, J.; Hartl F. U.; Hayer-Hartl, M.; Radford, S. E. J Biol Chem 2002, 277, 33115–33126.
104. Miyazaki, T.; Yoshimi, T.; Furutsu, Y.; Hongo, K.; Mizobata, T.; Kanemori, M.; Kawata, Y. J Biol Chem 2002, 277, 50621–50628.
105. Nojima, T.; Murayama, S.; Yoshida, M.; Motojima, F. J Biol Chem 2008, 283, 18385–18392.
106. Rye, H. S.; Roseman, A. M.; Chen, S.; Furtak, K.; Fenton, W. A.; Saibil, H. R.; Horwich, A. L. Cell 1999, 97, 325–338.
107. Ranson, N. A.; Clare, D. K.; Farr, G. W.; Houldershaw, D.; Horwich, A. L.; Saibil, H. R. Nat Struct Mol Biol 2006, 13, 147–152.
108. Llorca, O.; Carrascosa, J. L.; Valpuesta, J. M. J Biol Chem 1996, 271, 68–76.
109. Sameshima, T.; Ueno, T.; Iizuka, R.; Ishii, N.; Terada, N.; Okabe, K.; Funatsu, T. J Biol Chem 2008, 283, 23765–23773.
110. Sameshima, T.; Iizuka, R.; Ueno, T.; Funatsu, T. Biochem J 2010, 427, 247–254.
111. Takei, Y.; Iizuka, R.; Ueno, T.; Funatsu, T. J Biol Chem 2012, 287, 41118–41125.
112. Mendoza, J. A.; Rogers, E.; Lorimer, G. H.; Horowitz, P. M. J Biol Chem 1991, 266, 13044–13049.
113. Fenton, W. A.; Kashi, Y.; Furtak, K.; Horwich, A. L. Nature 1994, 371, 614–619.
114. Elad, N.; Farr, G. W.; Clare, D. K.; Orlova, E. V.; Horwich, A. L.; Saibil, H. R. Mol Cell 2007, 26, 415–426.
115. Farr, G. W.; Fenton, W. A.; Chaudhuri, T. K.; Clare, D. K.; Saibil, H. R.; Horwich, A. L. EMBO J 2003, 22, 3220–3230.
116. Farr, G. W.; Furtak, K.; Rowland, M. B.; Ranson, N. A.; Saibil, H. R.; Kirchhausen, T.; Horwich, A. L. Cell 2000, 100, 561–573.
117. Falke, S.; Tama, F.; Brooks, C. L., III; Gogol, E. P.; Fisher, M. T. J Mol Biol 2005, 348, 219–230.
118. Clare, D. K.; Bakkes, P. J.; van Heerikhuizen, H.; van der Vies, S. M.; Saibil, H. R. Nature 2009, 457, 107–110.
119. Horst, R.; Bertelsen, E. B.; Fiaux, J.; Wider, G.; Horwich, A. L.; Wüthrich, K. Proc Natl Acad Sci USA 2005, 102, 12748–12753.
120. Koculi, E.; Horst, R.; Horwich, A. L.; Wüthrich, K. Protein Sci 2011, 20, 1380–1386.
121. Lin, Z.; Rye, H. S. Mol Cell 2004, 16, 23–34.
122. Buckle, A. M.; Zahn, R.; Fersht, A. R. Proc Natl Acad Sci USA 1997;94:3571–3575.
123. Chen, L.; Sigler, P. B. Cell 1999, 99, 757–768.
124. Wang, Z.; Feng, H. P.; Landry, S. J.; Maxwell, J.; Gierasch, L. M. Biochemistry 1999, 38, 12537–12546.
125. Chen, S.; Roseman, A. M.; Hunter, A. S.; Wood, S. P.; Burston, S. G.; Ranson, N. A.; Clarke, A. R.; Saibil, H. R. Nature 1994, 371, 261–264.

126. Thiyagarajan, P.; Henderson, S. J.; Joachimiak, A. Structure 1996, 4, 79–88.
127. Clare, D. K.; Bakkes, P. J.; van Heerikhuizen, H.; van der Vies, S. M.; Saibil, H. R. J Mol Biol 2006, 358, 905–911.
128. van Duijn, E.; Simmons, D. A.; van den Heuvel, R. H.; Bakkes, P. J.; van Heerikhuizen, H.; Heeren, R. M.; Robinson, C. V.; van der Vies, S. M.; Heck, A. J. J Am Chem Soc 2006, 128, 4694–4702.
129. van Duijn, E.; Heck, A. J.; van der Vies, S. M. Protein Sci 2007, 16, 956–965.
130. Shtilerman, M.; Lorimer, G. H.; Englander, S. W. Science 1999, 284, 822–825.
131. Lin, Z.; Madan, D.; Rye, H. S. Nat Struct Mol Biol 2008, 15, 303–311.
132. Motojima, F.; Chaudhry, C.; Fenton, W. A.; Farr, G. W.; Horwich, A. L. Proc Natl Acad Sci USA 2004, 101, 15005–15012.
133. Ditzel, L.; Löwe, J.; Stock, D.; Stetter, K. O.; Huber, H.; Huber, R.; Steinbacher, S. Cell 1998, 93, 125–138.
134. Gupta, R. S. Biochem Int 1990, 20, 833–841.
135. Klumpp, M.; Baumeister, W.; Essen, L. O. Cell 1997, 91, 263–270.
136. Dekker, C.; Roe, S. M.; McCormack, E. A.; Beuron, F.; Pearl, L. H.; Willison, K. R. EMBO J 2011, 30, 3078–3090.
137. Kalisman, N.; Adams, C. M.; Levitt, M. Proc Natl Acad Sci USA 2012, 109, 2884–2889.
138. Kusmierczyk, A. R.; Martin, J. Biochem J 2003, 371, 669–673.
139. Schoehn, G.; Hayes, M.; Cliff, M.; Clarke, A. R.; Saibil, H. R. J Mol Biol 2000, 301, 323–332.
140. Clare, D. K.; Stagg, S.; Quispe, J.; Farr, G. W.; Horwich, A. L.; Saibil, H. R. Structure 2008, 16, 528–534.
141. Booth, C. R.; Meyer, A. S.; Cong, Y.; Topf, M.; Sali, A.; Ludtke, S. J.; Chiu, W.; Frydman, J. Nat Struct Mol Biol 2008, 15, 746–753.
142. Huo, Y.; Hu, Z.; Zhang, K.; Wang, L.; Zhai, Y.; Zhou, Q.; Lander, G.; Zhu, J.; He, Y.; Pang, X. Structure 2010, 18, 1270–1279.
143. Zhang, J.; Baker, M. L.; Schröder, G. F.; Douglas, N. R.; Reissmann, S.; Jakana, J.; Dougherty, M.; Fu, C. J.; Levitt, M.; Ludtke, S. J.; Frydman, J.; Chiu, W. Nature 2010, 463, 379–383.
144. Zhang, J.; Ma, B.; DiMaio, F.; Douglas, N. R.; Joachimiak, L. A.; Baker, D.; Frydman, J.; Levitt, M.; Chiu, W. Structure 2011, 19, 633–639.
145. Cong, Y.; Schröder, G. F.; Meyer, A. S.; Jakana, J.; Ma, B.; Dougherty, M. T.; Schmid, M. F.; Reissmann, S.; Levitt, M.; Ludtke, S. L.; Frydman, J.; Chiu, W. EMBO J 2011, 31, 720–730.
146. Reissmann, S.; Joachimiak, L. A.; Chen, B.; Meyer, A. S.; Nguyen, A.; Frydman, J. Cell Rep 2012, 2, 866–877.
147. Kusmierczyk, A. R.; Martin, J. FEBS Lett 2003, 547, 201–204.
148. Bigotti, M. G.; Clarke, A. R. J Mol Biol 2005, 348, 13–26.

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