FEBS Letters 589 (2015) 2522–2532

journal homepage: www.FEBSLetters.org

Review

Dynamics, flexibility, and allostery in molecular chaperonins

Lars Skjærven [a,*], Jorge Cuellar [b], Aurora Martinez [a], José María Valpuesta [b]

[a] Department of Biomedicine, University of Bergen, Bergen, Norway
[b] Department of Macromolecular Structure, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain

ARTICLE INFO

Article history:
Received 10 May 2015
Revised 18 June 2015
Accepted 23 June 2015
Available online 30 June 2015

Edited by Wilhelm Just

Keywords:
Chaperonin
Chaperone
Protein folding
Allostery
Conformational changes
Protein dynamics

ABSTRACT

The chaperonins are a family of molecular chaperones present in all three kingdoms of life. They are classified into Group I and Group II. Group I consists of the bacterial variants (GroEL) and the eukaryotic ones from mitochondria and chloroplasts (Hsp60), while Group II consists of the archaeal (thermosomes) and eukaryotic cytosolic variants (CCT or TRiC). Both groups assemble into a dual ring structure, with each ring providing a protective folding chamber for nascent and denatured proteins. Their functional cycle is powered by ATP binding and hydrolysis, which drives a series of structural rearrangements that enable encapsulation and subsequent release of the substrate protein. Chaperonins have elaborate allosteric mechanisms to regulate their functional cycle. Long-range negative cooperativity between the two rings ensures alternation of the folding chambers. Positive intra-ring cooperativity, which facilitates concerted conformational transitions within the protein subunits of one ring, has only been demonstrated for Group I chaperonins. In this review, we describe our present understanding of the underlying mechanisms and the structure–function relationships in these complex protein systems with a particular focus on the structural dynamics, allostery, and associated conformational rearrangements.

1. Introduction

Most proteins require assistance to fold into their three-dimensional native state and achieve their functional activity [1]. This is due to the extremely crowded environment within the cell, which renders newly synthesized proteins prone to form toxic aggregate species. Given the need to minimize aggregation, nature has developed quality control mechanisms, including a complex system of chaperone surveillance that ensures protein homeostasis, or proteostasis [2,3]. The chaperonins, a critical group of molecular chaperones, are large double-ring complexes of 800–1000 kDa built of 7–9 subunits per ring (Table 1). The chaperones in this family facilitate protein folding by providing a protective chamber where non-native substrate proteins can enter and (re)-fold, in isolation from the cell environment to avoid destructive molecular interactions. To enable encapsulation and subsequent release of the substrate protein, chaperonins undergo a series of ATP-dependent conformational transitions [4,5].

Chaperonins are classified in two distantly related structural groups (Table 1); Group I is found in bacteria (GroEL; from growth essential large) and eukaryotic organelles (Hsp60; heat-shock protein 60), while Group II is expressed in Archaea (thermosome) and in the eukaryotic cytosol (chaperonin containing TCP1 (CCT), or TCP1 ring complex (TRiC)). Their gene family is extensive and complex [6–8], but the overall architecture is largely conserved. The main structural difference between Groups I and II is the lid arrangement that seals off the central chamber (Fig. 1). Group I chaperonins cooperate with Hsp10 (GroES in *Escherichia coli*), which provides a lid that covers the folding chamber to create the closed conformation, whereas in the Group II variants, the lid that seals the central chamber is formed by a built-in unit made of a long α-helix attached to the apical domain in each subunit (Fig. 1). Despite the differences in lid arrangement and the divergent amino acid sequences, which show pairwise identity of only ~20% (Fig. S1), their structural similarity at the subunit and oligomeric levels is striking (Fig. 1).

The main steps in the reaction cycle have been well established over the last two decades by extensive functional and structural studies including X-ray crystallography [9,10], electron microscopy (EM) [11,12], and to a lesser degree, NMR [13] and SAXS [14]. These tools have been decisive in determining the structure of the chaperonins in several states along their functional cycle. The exponential increase in computational power during the last decade has opened for extensive simulation studies providing a more

* Corresponding author.
E-mail address: lars.skjarven@uib.no (L. Skjærven).

http://dx.doi.org/10.1016/j.febslet.2015.06.019
0014-5793/

Table 1
Chaperonin classification (adapted from [8]).

Occurrence	Group I		Group II	
	Bacteria	Eukaryotic organelles	Archaea	Eukaryotic cytosol
Name	GroEL	Hsp60	Thermosome	CCT (TRiC)
Co-chaperone	GroES	Hsp10	–	–
# Subunit types	1	1	1–3	8
Oligomerization	2 × 7	2 × 7	2 × 8/2 × 9	2 × 8

complete view of possible trajectories between the stable end-states, transient conformations, and detailed mechanisms underlying ligand-induced conformational transitions [15]. In this review, we present an overview of the structure–dynamics–function relationships in this class of molecular chaperones. We start by surveying current knowledge of the GroEL functional cycle, with associated conformational transitions and models of allostery. We then present the less-characterized Group II chaperonins, the thermosome and CCT.

2. Group I chaperonins: GroEL–GroES

2.1. Overall architecture

GroEL is an oligomer composed of two chemically identical homoheptameric rings stacked back-to-back [9,16,17]. In its open, substrate-receptive state, the two rings form a ~150 Å-long cylindrical structure with a diameter of ~145 Å (Fig. 1A) [9,18]. At each end of the cylinder, the structure forms a ~45 Å deep and wide cavity that constitutes the folding chamber [9]. In the *folding active* state, the chamber is extended to a ~55 Å wide and ~80 Å deep cavity capped by the co-chaperone GroES (which is also a ring-shaped heptameric structure; Fig. 1A). This provides a confined, protective hydrophilic environment in which non-native substrate proteins can refold without inappropriate interactions in the crowded cell environment [16,19,20].

The GroEL monomer has 547 residues and folds into three distinct structural domains: the *equatorial*, *intermediate*, and *apical* domain (Fig. 1A). The equatorial is a solid α-helical domain that provides most intra-ring subunit contacts and all inter-ring contacts [9,16] (Fig. 1A). It also contains the nucleotide binding site which is located at its top, facing the adjacent subunit [16]. The apical domain is situated at the end of the cylinder and forms the entrance to the folding chamber. It contains exposed hydrophobic residues responsible for interactions with non-native substrate proteins, as well as a number of charged residues that facilitate inter-subunit salt bridges. The intermediate domain acts as a hinge between the apical and equatorial domains, providing flexibility to the GroEL assembly and facilitating large-scale conformational changes [16].

2.2. Allostery

GroEL-assisted protein folding is precisely regulated by ATP binding and hydrolysis, the main facilitators of the large-scale structural changes responsible for the cycling between substrate folding and release states [21]. The high cooperativity of ATP binding and hydrolysis in the GroEL/ES system, revealed by early functional and kinetic studies [22–24], was later associated to

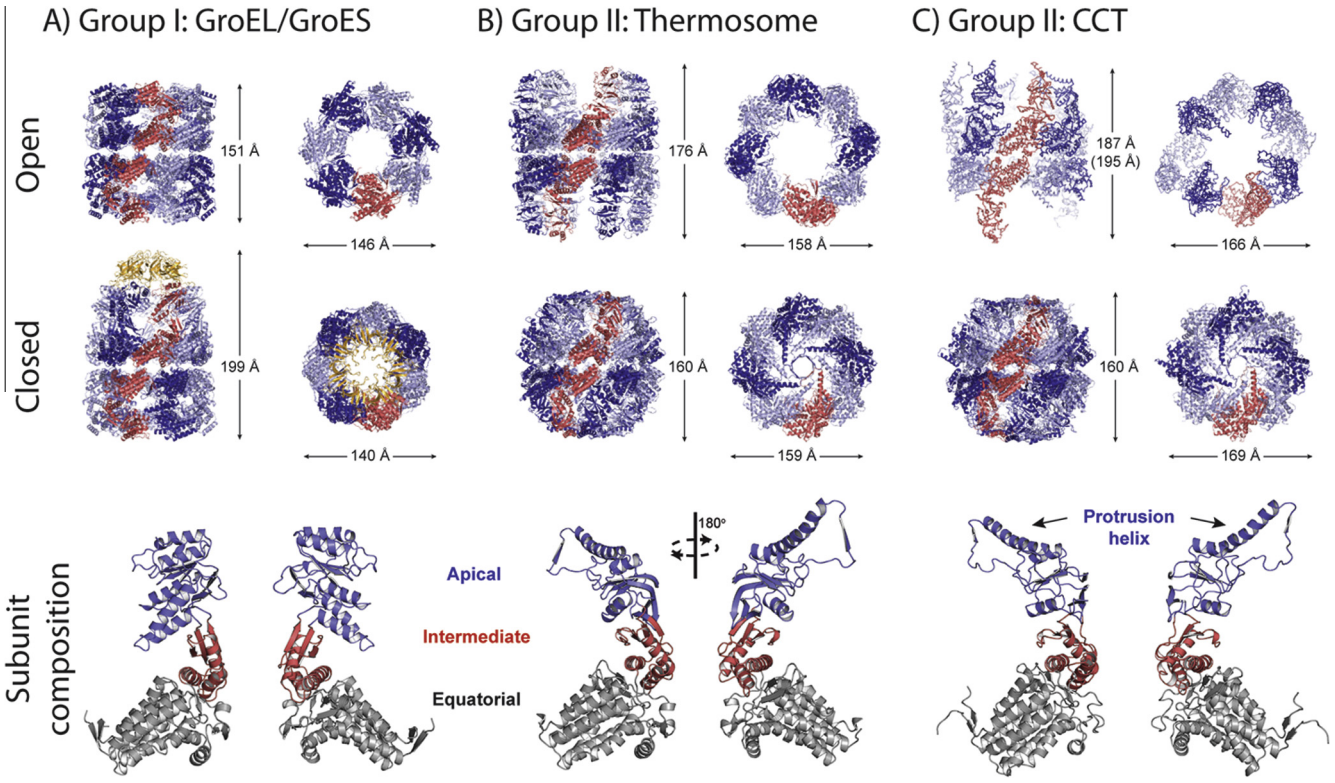

Fig. 1. Overview of chaperonin structures. Major conformational species of Group I and II chaperonins are shown in cartoon representation. (A) GroEL/ES, (B) thermosome, and (C) CCT are shown in columns one to three, respectively. The individual subunits are coloured alternating dark and light blue, and the co-chaperone GroES is shown in orange. Two inter-ring adjacent subunits are highlighted in red colour to illustrate the interaction relationship between the rings (1:2 for GroEL, and 1:1 for thermosomes and CCT). Closed (folding active) and open (folding inactive) structures are shown in rows one and two, respectively. The atomic structures of individual subunit structures (closed form) are shown in the third row from two angles. The subunit is colored according to their domain composition: blue (apical domain), red (intermediate domain), and grey (equatorial domain). The protrusion helix of CCT is labelled to indicate the dominating structural deviation between Group I and II. PDB codes: GroEL/ES: 1XCK (open) and 1SX4 (closed); thermosome: 3KFK (open, Δlid) and 1A6D (closed); CCT: 2XSM (open) and 4V8R (closed). Reported dimensions are calculated from the coordinates provided in the respective PDB structure and might deviate from those reported in their original paper.

structural changes [25]. Subsequent analyses described both positive (within a ring) and negative cooperativity (between rings) [17,20,26,27]. Interestingly, the positive intra-ring cooperativity has been shown to be present only for ATP. ADP and ATP analogues on the other hand bind in a non-cooperative manner [28], and thus the role of the γ-phosphate of ATP has been shown to be critical in inducing the allosteric interactions in GroEL [29].

The classic Monod–Wyman–Changeux (MWC) model of allostery assumes that ligand-induced conformational changes affect mainly the quaternary structure of the oligomeric complex, and consist of concerted rotations of the subunits that maintain symmetry [30]. According to the alternative sequential Koshland–Nemethy–Filmer (KNF) model the subunits change conformation one at a time after ligand binding [31]. For GroEL/ES, it is now clear that neither model alone can describe the allosteric mechanism, and a model for *nested cooperativity* in GroEL was postulated [27]. This model combines MWC and KNF; GroEL intra-ring cooperativity is represented by the MWC model, while inter-ring negative cooperativity follows KNF type transitions (for review, see [32]).

2.3. Conformational changes and reaction cycle

GroEL undergoes a series of conformational transitions that govern and drive the functional cycle of productive protein folding [33–35]. The cycle is powered by ATP binding and hydrolysis, and is regulated by the complex interplay of positive and negative cooperativity [22,27]. The main steps of the reaction cycle have been established by extensive structural and functional studies over the last two decades. The cycle of events can be summarized in four major steps, which entail (1) binding of ATP and substrate protein to one ring of the GroEL assembly, (2) GroES binding and encapsulation of the substrate protein, (3) ATP hydrolysis, and (4) ATP binding to the opposite ring, with subsequent release of the ligands from the first ring (Fig. 2A). A new cycle is initiated by ATP binding in the fourth step, and the previously inactive ring becomes the new folding active ring for the next iteration of productive folding. We describe these main steps in more detail below, focusing on the structural mechanisms for allosteric interactions and conformational changes.

2.3.1. ATP binding

The GroEL functional cycle is initiated by cooperative binding of seven ATP molecules to the equatorial domains within the same ring (denoted *cis*). This triggers the first large-scale conformational events in the two-stage transition to the fully extended GroEL/ES structure, whereas there are only minor changes in the opposite, folding inactive ring (denoted *trans*) [38]. This first step entails concerted movements of individual domains in the *cis* ring to produce an intermediate, semi-relaxed (**R1**) state that is slightly expanded compared to the unliganded, tense (**T**) state [12,34,38]. That these motions must be concerted was first appreciated by early computational studies, which showed that non-concerted motions would impede transition due to steric intra-ring clashes [39,40]. It thus appears that GroEL undergoes *coupled tertiary structural changes* [40] rather than the quaternary transition found in many other allosteric proteins [32].

This initial conformational change after ATP binding most notably involves the apical domains, which undergo a 25° counterclockwise twist (as seen from above the cavity) with a moderate elevation (Fig. 3A). In addition, the equatorial domains produce an 8° tilt and the intermediate domains rotate downwards about 20°. This results in helix M covering the nucleotide binding site of the equatorial domain (Fig. 3A), in which is proposed to act as a conformational trigger for the subsequent larger movements of the apical and equatorial domains [39].

Cryo-EM studies showed that these transitions are associated with a series of salt bridge breaking and switching events between adjacent domains, which would free the apical and intermediate domains and facilitate conformational transitions (Fig. 3B). The fluctuating salt-bridge interactions, visible in recently reported EM densities [12], involve residues that are conserved in all GroEL sequences, thus highlighting their functional importance [41]. At the intra-ring level, the R197-E386 salt bridge between the intermediate and the apical domains of two adjacent subunits that is found in the unliganded (apo) state is replaced by K80-E386 in the ATP-bound state [34], whereas E255-K207 switches to E255-K245 in the ATP-bound state (Fig. 3B) [12]. Results from EM studies indicate that both of these newly formed salt bridges are broken during a second intermediate transition on the path to the GroES bound state [12]. In molecular dynamics (MD) simulation studies, the D83-K327 interaction within each subunit ruptures rapidly after ATP binding, which is thought to be coupled with inter-subunit R197-E386 salt bridge breakage [42,43].

Despite this extensive structural knowledge, the detailed mechanisms that explain how nucleotide binding to the equatorial domains drives these events remain largely unknown. Computational analyses of 280 subunit structures (available from the Protein Data Bank) with complementing MD simulations suggest the presence of two sub-domains within the equatorial domain, and that ATP binding triggers both local and global structural changes within the domain (Fig. 3C) resulting in an initial stabilization [42]. A set of unique atomic interactions takes place, which notably involves the formation of a salt bridge between K34-E483 adjacent to the ATP-binding site, and a disruption of the L134-N475 interaction on the opposite side of the nucleotide binding site [44]. MD simulations showed significantly reduced interaction energies between several charged inter-subunit residue contacts following binding of ATP to the *cis* ring. A concomitant opening of the subunits with elevated apical domains was also observed, which indicates that ATP association causes rapid inter-subunit destabilization, which enables subsequent larger scale transitions [44].

Cryo-EM studies further showed an altered interaction pattern at the inter-ring interface after ATP binding [34,38], which is interpreted as the main mechanism for negative inter-ring cooperativity. Helix D, located in the equatorial domain, couples the ATP binding site of a subunit in one ring to an adjacent subunit of the opposite ring (Fig. 3D). In the unliganded (**T**) state, two adjacent helices D are aligned whereas after ATP binding, the two helices reorient to a partially offset position, and further to a fully offset position after GroES binding [38]. Due to this apparent coupling between two distant nucleotide binding sites and its suggested role as a mediator of negative cooperativity, helix D has been dubbed the *relay helix* [38].

Simulations of the GroEL oligomer starting from the unliganded conformational state, showed on the contrary that two adjacent helices D produce a full offset motion when introducing ATP to one of the rings [44]. This offset motion, which was not observed in simulations under the same conditions but without ATP, resembles the offset in the GroEL/ES complex and is congruent with the rapid transmission of negative cooperativity between the rings (Fig. 3D). The simulations also showed that reorientation of helices D is coupled to formation of an inter-ring salt bridge between E434 and R430 [44]. The importance of this interaction in inter-ring communication is supported by mutational studies showing that inter-ring allostery is decreased in the GroEL E434K variant [45,46]. Further MD simulations of this mutant showed no displacement of helices D as a response to ATP-binding, even though the *cis* ring appeared to undergo the same conformational changes as seen in simulations of wild-type GroEL.

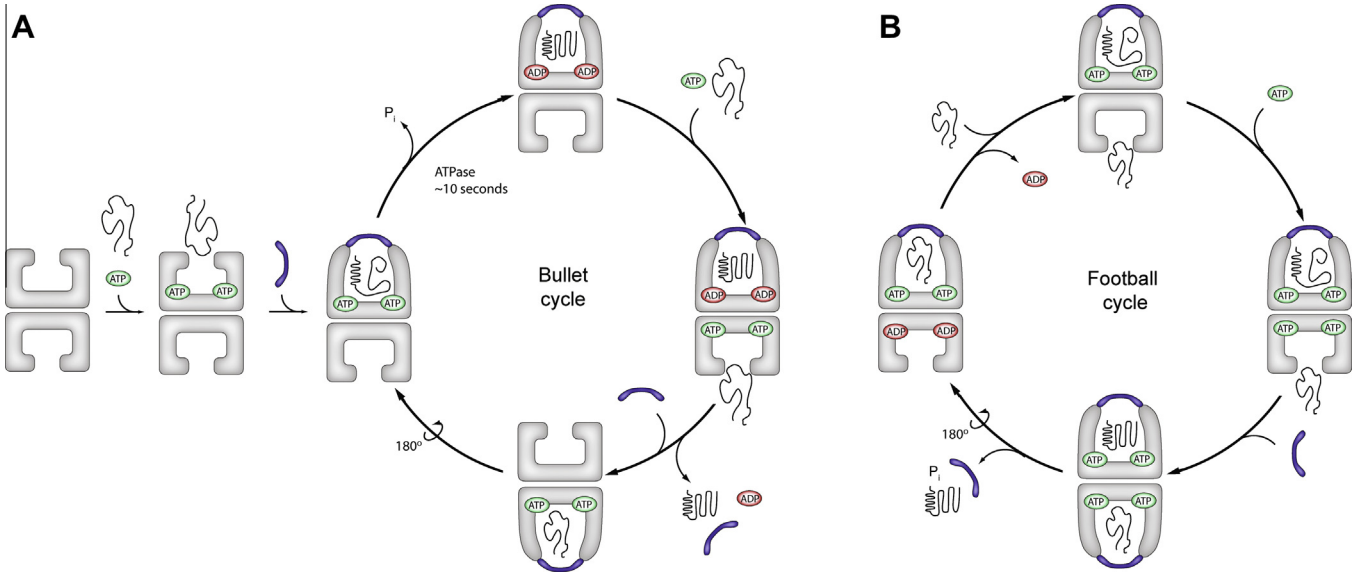

Fig. 2. GroEL/ES reaction cycle. Two primary views on the reaction cycle of the GroEL–GroES chaperonin. (A) The traditional model termed the "bullet cycle" assumes that only one folding chamber is active at a given time. ATP- and substrate binding facilitates GroES binding resulting in an encapsulated folding chamber (the *cis* ring). ATP hydrolysis enables ATP binding in the opposite *trans* ring which triggers the discharge of ligands from the cis ring, and a new cycle of GroEL mediated folding is initiated [12]. (B) The "football cycle" assumes that the GroEL–GroES$_2$ complex is the predominate species in the presence of substrate proteins, while GroEL–GroES$_1$ predominates in the absence of substrate proteins [36,37].

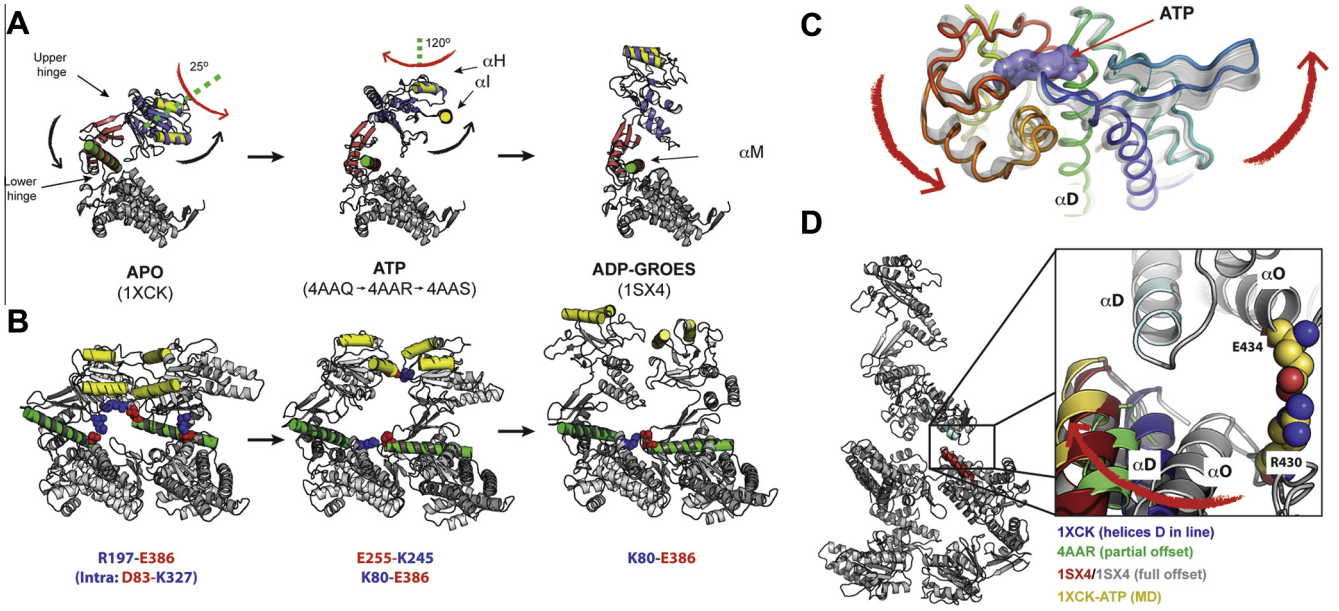

Fig. 3. Subunit flexibility in GroEL. (A) The GroEL subunit undergoes large-scale conformational changes along the reaction cycle. Upon ATP-binding the apical domain (blue) rotates upwards while helix M (green cylinder) in the intermediate domain (red) moves down to cover over the nucleotide binding site. GroES association triggers a rotation of the apical domain in the opposite direction, and is associated with an additional upward motion. This produces the end state where the exposed hydrophobic residues in helices H + I (yellow cylinders) are hidden from the folding chamber. (B) The conformational transitions are associated to a series of salt bridge formation and breakage at the inter-subunit level. (C) Principal component analysis of all available structures of the equatorial domain shows intra domain conformational changes associated with nucleotide binding. (D) One subunit from the folding active ring interacts with two subunits from the opposite ring. The inset zoom shows two adjacent helices D at different conformational states along the folding cycle. Two adjacent helices D are initially in-line (unliganded state; PDB ID: 1XCK). Upon ATP binding the two helices D are shifted to an offset position (1XCK-ATP MD). The MD simulations (yellow) suggest a strong interaction between E434 and R430.

2.3.2. Substrate association

Whether protein substrate or ATP binding comes first in the reaction cycle is still debated. It was long suggested that substrate binding precedes ATP binding and that, with associated apical domain movements, ATP binding has a role in protein unfolding; movements of the apical domains to which the protein substrate is attached force protein unfolding [47]. New data nonetheless suggest that ATP binding is more rapid than substrate association, indicating that ATP might already be bound when the substrate protein interacts with GroEL [48]. ATP addition does not appear to enhance substrate protein stretching, which would occur in a forced unfolding mechanism [48]. In contrast, the variety of

conformations attained by the apical domains in the presence of ATP [12,44] could facilitate substrate binding by supplying more complementary surfaces than a rigid GroEL structure in the **T** state could provide.

Recent EM studies captured the structure of GroEL with bound substrate in open [49,50] and closed [51,52] ring conformations. The EM densities of non-native malate dehydrogenase bound to GroEL (open conformation) suggest several possible binding topologies, ranging from deep inside the cavity to the cavity inlet, where the substrate seems to be restricted to contacting three or four of the seven consecutive GroEL apical domains (Fig. 4A) [49]. The binding mode is similar for the larger viral capsid protein gp23, which contacts at least five of the seven apical domains within the ring [50]. These studies also show that the sevenfold rotational symmetry within a ring is disrupted when accommodating the substrate protein, and the associated apical motions are suggested to represent the main conformational effects on substrate binding to GroEL [51,52], a similar behavior to that found in CCT [11] (see below).

2.3.3. GroES binding and substrate folding

The GroEL oligomer in the ATP-bound state with elevated and rotated apical domains is able to bind the co-chaperone GroES [53]. This association triggers a new set of rigid body domain movements involving a 120° clockwise rotation of the apical domain (as compared to the ATP-bound state), producing a stable end state of GroEL/ES (the **R2** state) with a large dome-shaped cavity [16]. During this transition, the hydrophobic substrate binding sites switch from interacting with the substrate to interacting with a portion of the GroES mobile loop at each GroES subunit, which orders as β-hairpins upon the complex formation. Besides providing an encapsulating lid for the cavity, GroES binding seems to be a prerequisite for triggering these large-scale transitions in GroEL. Throughout this association with GroES, the polypeptide substrate is released from its hydrophobic binding sites at the apical domains to the newly generated folding chamber, whose volume is now ~120,000 Å^3. In this state, the cavity walls create a highly hydrophobic environment, which effectively minimizes exposure of the substrate protein's hydrophobic residues [50].

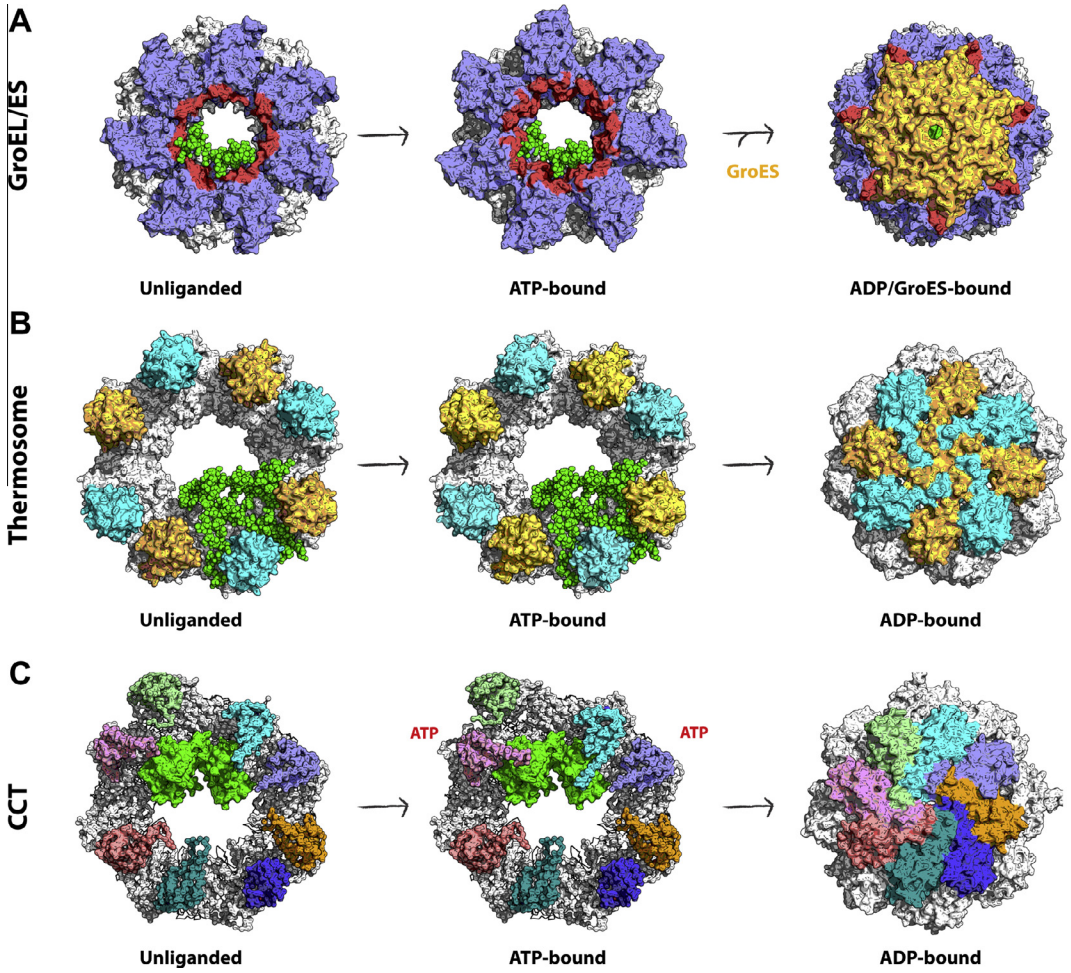

Fig. 4. Models of ATP-dependent folding mechanisms (adapted from Ref [4]). (A) GroEL in its open substrate-receptive conformation (left) recognizes and traps unfolded proteins (green) through hydrophobic residues located in the apical domains (blue), at the entrance of the cavity (helices H + I; red). Concerted ATP binding in all seven subunits induces conformational changes (centre). (Right) ATP binding facilitates association of the co-chaperonin GroES (yellow), that caps the cavity and induces enlargement of the cavity while the unfolded protein is released into the chamber, where folding takes place. (B) The thermosome also has an open conformation (left) that recognizes and traps unfolded polypeptides using a hydrophobic mechanism similar to that of GroEL. (Centre) ATP binding and hydrolysis induces closure of the cavity. (Right) This is performed by a helical lid inserted in the apical domain. Closure of the cavity would release the substrate into the chamber, where it could fold. (C) In the case of CCT, specific proteins interact through defined regions with specific CCT subunits (left) in the open substrate-receptive conformation. (Centre) ATP binding to certain subunits triggers a non-concerted ATP hydrolysis mechanism. (Right) This results in the closure of the cavity and the holding of the substrate without being released in the cavity. PDB IDs of shown structures: GroEL: 1XCK, 4AAR, 1SX4; Thermosome: 3KFK and 1A6D; CCT: 2XSM and 4V8R. The equatorial and intermediate domains are colored light grey, while models of the substrate protein is colored green in all structures.

The chamber is also proposed to limit the accessible conformational space of the substrate protein in the folding process, and to prevent aggregation and contacts with any other protein in the crowded cell milieu [54].

EM densities of unfolded rubisco encapsulated within the GroEL/ES assembly show that the normally unstructured C-terminal tails of the GroEL subunits stretch from the equatorial region to establish numerous interactions with the substrate protein [52]. The substrate also finds interaction points at the lower segments of two of the seven apical domains [52]. Other EM studies of fully folded substrate proteins suggest repulsion towards the cavity wall [12,55]. The presence of substrate within the GroEL cavity also adjusts cavity shape to accommodate the substrate [55,56].

2.3.4. ATP hydrolysis and binding to the opposite ring

ATP hydrolysis occurs ~4–20 s after association, and is the main determinant for ATP binding to the opposite, *trans* ring [57–59]. ATP hydrolysis is thought to help weaken the interaction towards GroES, which facilitates subsequent ligand release [38,60]. Whereas the structural rearrangements of adjacent helices D at the inter-ring interface (see above) appear to be a major facilitator of inter-ring communication, it remains unknown how the mechanisms that underlie ATP binding in *trans* trigger *cis* ligand release. Simulation studies of the bullet-shaped GroEL/ES-ADP complex without ATP or with ATP bound in the *trans* ring suggest that ATP binding reverses the effects of nucleotide binding in the *cis* ring; the two helices D shift back to an aligned position during the relatively short simulations. This provides a hypothesis for the initial structural mechanisms for *cis* ligand release [44].

ATP binding to an open *trans* ring in the asymmetric bullet complex not only facilitates ejection of the cis ligands, but also initiates a new cycle in which the folding inactive ring becomes the active ring (Fig. 2). The two rings thus alternate the job as folding facilitators, working out of phase with each other. The energy released by ATP binding drives the large-scale transitions, while ATP hydrolysis acts as a timer and provides directionality for the folding cycle [61].

2.3.4.1. Symmetric football-shaped assemblies. Although the main elements of the reaction cycle have been established through numerous structural and functional studies, controversy continues to surround the precise stoichiometry of the complex, with important implications for understanding of the GroEL/ES reaction cycle. In the prevalent view, the two folding chambers work alternately like a two-stroke engine (Fig. 2A), with only one folding chamber active at any given time [58,60–62]. In this model, the asymmetric GroEL–GroES$_1$ state (Fig. 1A) is the predominant species, due in part to the long-lived ATP-bound state (~10 s).

This model was nonetheless challenged by the observation of symmetric GroEL–GroES$_2$ complexes –the so called *football* complex (Fig. 2B) – in which GroES is attached to both sides of the GroEL cylinder [14,63–66]. These findings from structural studies such as EM and small-angle X-ray scattering are supported by kinetic analyses, and have recently given rise to an alternative proposal for the reaction cycle [36,37,67]. In this model (Fig. 2B), the symmetric GroEL–GroES$_2$ complex is suggested to be the predominant functional form [19], where the two folding chambers operate as a parallel processing machine rather than functioning alternately.

3. Group II chaperonins: thermosome and CCT

3.1. Overall architecture

The structure is more complex for Group II chaperonins. These include the archaeal chaperonins, commonly termed thermosomes, which are composed of octameric or nonameric rings made up of one, two or three different subunits [68]. The other Group II chaperonin is found in the cytosol of eukaryotic organisms, and is usually termed CCT (chaperonin containing TCP1) or TRiC (TCP1 ring complex) [4,69]. CCT is composed of eight distinct subunits (CCTα-1, CCTβ-2, CCTγ-3, CCTδ-4, CCTε-5, CCTζ-6, CCTη-7 and CCTθ-8) organized in a unique intra- and inter-ring arrangement that was recently determined, although the exact hand of the arrangement has not been resolved [70,71].

Recent years have witnessed a great deal of structural information derived from X-ray and EM data [10,11,72–78] (Fig. 1B and C). From these and other studies, we can conclude that Group II chaperonins share a common monomer domain structure with Group I, that is, they have an equatorial, intermediate and apical domain. Both groups also show similar conformational states: an open, substrate-receptive conformation in which the unfolded protein is recognized, and a closed, folding active conformation. There are nonetheless major differences as to how the closed conformation is acquired, since the Group I co-chaperonin is replaced in Group II by an extra helix (helical protrusion) at the tip of the apical domain (Fig. 1). Another important difference is the way in which the two rings interact; Group I chaperonins show a 1:2 subunit arrangement, whereas Group II shows an in-phase 1:1 arrangement (Fig. 1). These structural features point to a divergent inter-ring signaling mechanism for the two chaperonin groups.

3.2. Allostery

Like Group I, Group II chaperonins have a set of complex intra- and inter-ring allosteric signals [21]. Although positive intra-ring cooperativity remains an open question, there is evidence of a non-concerted mechanism, in particular for CCT [74,79–82]. In contrast to Group I chaperonins, the open conformation structures of thermosome/CCT show no impediment to non-concerted intra-ring conformational changes of individual subunits. There are no lateral contacts between intermediate or apical domains of adjacent subunits (Fig. 5B), and these contacts occur at only two places in the equatorial domain [11,73,74]. The first contact is between loops H5/H6 of one subunit and loop H16/H17 of an adjacent subunit, and is proposed to be a key point for allosteric regulation (Fig. 5A and B). The second contact takes place through a β-sheet formed by the N and C termini of one subunit and β-strands S2 and S3 of the neighboring subunit. This region undergoes large conformational changes between the open and closed states (Fig. 5B) [76].

Although a KNF model has been described for inter-ring signaling in the two chaperonin groups [83], there are differences between them, probably linked to the variations in inter-ring structure described above. ATP saturation of the two rings in Group I results in decreased ATP hydrolysis [26], probably due to steric clash caused by simultaneous ATP hydrolysis in both. This is not the case for Group II, which show a higher ATP hydrolysis rate, which indicates no inter-ring interference [80,82,84,85].

3.3. Conformational changes and reaction cycle

Group II chaperonins cycle between an open, substrate-receptive conformation and a closed, substrate-trapping conformation in a manner similar to that of Group I. Structures of thermosome in the open state show that the structural integrity of the inter-ring interface relies mostly on hydrophobic interactions (Fig. 4B) [73,74]. In the case of eukaryotic CCT, the crystal structure of the open conformation reveals a highly asymmetric structure, in which the apical domains of the eight subunits each adopt distinct conformations within the ring [11].

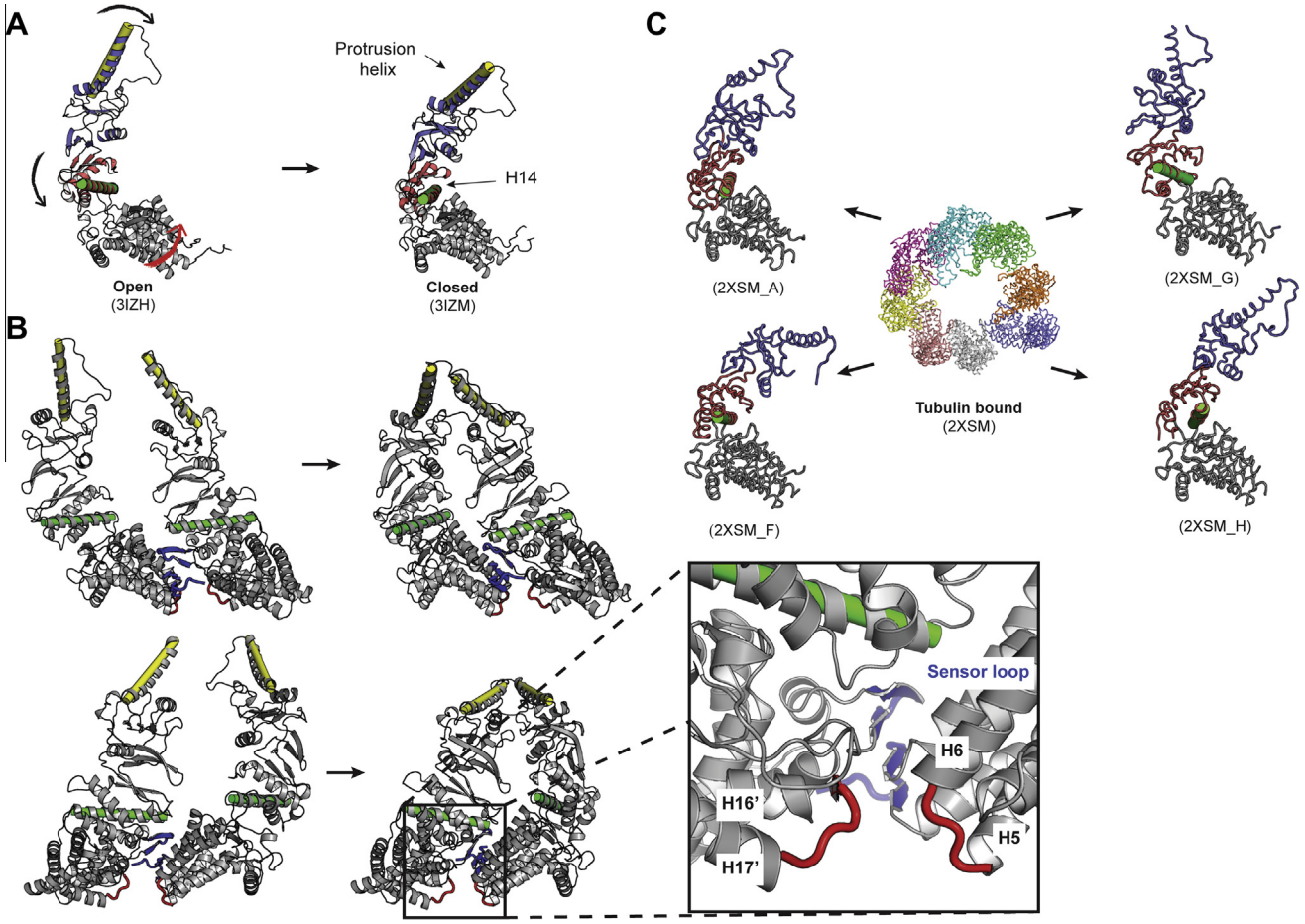

Fig. 5. Subunit flexibility of Group II chaperonins. (A) ATP-dependent transitions of thermosome at the monomeric level. The subunit of Group II chaperonins shows a downward tilt of the apical domain (blue), connected with a downward rotation of helix 14 (H14; green) in the intermediate domain upon ATP binding. The equatorial domain experiences a ~35° tilt. (B) Structural transitions at the dimer level highlighting the sensor loop (blue), as well as loops between H5/H6 and H16/H17 (red). The inset shows a zoom of the subunit interface at the equatorial domain. (C) Conformational heterogeneity of CCT (PDB ID 2XSM).

High-resolution structures of the closed conformation have been determined for several Group II chaperonins and show the two rings closed [10,72,78,86]. This finding reinforces the concept that there are no steric impediments to simultaneous closure of both rings, a structural feature that could help to explain the kinetic behavior differences between the two chaperonin groups. An interesting characteristic of the Group II chaperonins is the role of the helical protrusion in the control of inter-ring communication [87,88]; the helices interact and stabilize the closed conformation [77,87]. Whereas the inter-ring region in Group I chaperonins shows considerable rigidity during the movement between closed and open states, that of the thermosomes undergoes large conformational changes, with the equatorial domains experiencing a ~35° tilt and breaking of several salt bridges that stabilize the closed conformation [89].

Although it follows a pattern of conformational movement similar to that of thermosomes, CCT is much more complex and is unique in several ways. First, unlike the rest of the chaperonins, CCT is not promiscuous, but seems to act on a limited, albeit large group of client proteins, in particular actin and tubulin [4,83,90]. Many other client proteins with very different native structures interact with CCT, so a common pattern of CCT-substrate interaction cannot be deduced. The only major CCT substrates that share a structural motif are a large number of proteins with WD40 domains, which need CCT assistance to acquire their final β-propeller structure [69,91,92].

The second important difference from the rest of the chaperonins is that, to achieve its functions, CCT is assisted by a large number of co-chaperones [4,83]. Prefoldin (PFD) is common to all Group II chaperonins (although the CCT-assisting PFD is also more complex than its archaeal counterpart; [93]); in addition, CCT interacts with the major chaperone Hsp70, which delivers substrates such as the Von Hippel-Lindau tumor suppressor protein (VHL) [94,95]. Other CCT co-chaperones are the phosducin-like proteins (PhLP), which assist the eukaryotic chaperonin in the folding of Gβ-transducin (aided by PhLP1) [96] as well as actin and tubulin (by PhLP2/3) [97]. Finally, CCT is assisted in the folding of α- and β-tubulin by another group of dedicated downstream chaperones termed tubulin cofactors (TBC), some of which appear to interact with the eukaryotic chaperonin [98].

The third difference between CCT and other chaperonins is its complexity (eight different subunits in the double-ring structure compared to 1–3 distinct subunits in other chaperonins), reflected in the conformational movements of the ring after ATP binding and hydrolysis. These changes are manifested in the asymmetric layout of the ATP binding sites in the open, substrate-bound [11] compared to the closed, substrate-unbound structures [72]. Whereas it is clear that these changes are concerted for Group I chaperonins, the mechanism in the eukaryotic chaperonin is more complex and functions in a hierarchical fashion (Fig. 4C) [79,81,99–101]. This was shown by genetic studies in *Saccharomyces cerevisiae* using mutants in the ATP-binding site of various CCT subunits [79] and

by cryo-EM analysis of CCT at different ATP concentrations [81]. These sequential movements are thought to be used by the eukaryotic chaperonin to fold substrates like actin and tubulin [102].

Several recent reports indicate that CCT has functional polarity, with two internal opposed faces with different properties [99–101]; these reports nonetheless differ in the composition of the two faces (Fig. 6). It is clear, however, that there is a *core* face formed by the contiguous subunits CCT5/CCT2/CCT4, in which the phenotypic effect of mutations in the ATP-binding site is severe [103]; these subunits have high ATP binding and hydrolysis activity. Another core face apposed to these subunits is formed by subunits CCT8/CCT6/CCT3, with no notable phenotypic effect caused by mutations in their ATP-binding site and which have low or negligible ATP binding and hydrolysis activities. The two remaining subunits, CCT1 and CCT7, are located between the two core faces and have intermediate properties. This CCT polarity suggests functional specialization, with one face dedicated to substrate recognition and the other to generation of movement, which is induced by ATP binding and hydrolysis and is necessary for the folding of chaperonin client proteins.

3.3.1. The substrate recognition mechanism

Although thermosomes use a non-specific, hydrophobic-based recognition mechanism similar to Group I, there are also differences [104], in particular those related to a region in the helical protrusion that is involved in substrate recognition (Fig. 4B) [73,74]. CCT is much more complex, as it has eight different apical domains; the substrate-binding region in each of these bears charged and hydrophilic residues in some subunits, whereas other subunits have hydrophobic residues [90,105]. This observation, together with data from several biochemical and structural studies, led to the hypothesis that the CCT:client protein interaction takes place through specific CCT subunits with specific domains of a misfolded protein that acquires a given conformation before interacting with CCT (Fig. 4C). Structural and biochemical information for different client proteins confirm this specificity [11,92,106], and in the case of the crystal structure for the CCT:tubulin complex [11], it shows another substrate-interacting region. This region, termed the *sensor loop*, is located in the equatorial domain at the tip of a β-hairpin formed by β-strands S2 and S3, which connects with the nearby ATP-binding pocket (Fig. 5C). The importance of this loop was confirmed in RNA interference experiments that show similar phenotypes for sensor loop mutants and ATP binding site mutants. This sensor loop lies near the N and C termini, which are also suggested to be involved in protein folding in GroEL and the thermosomes [56,76,107–109].

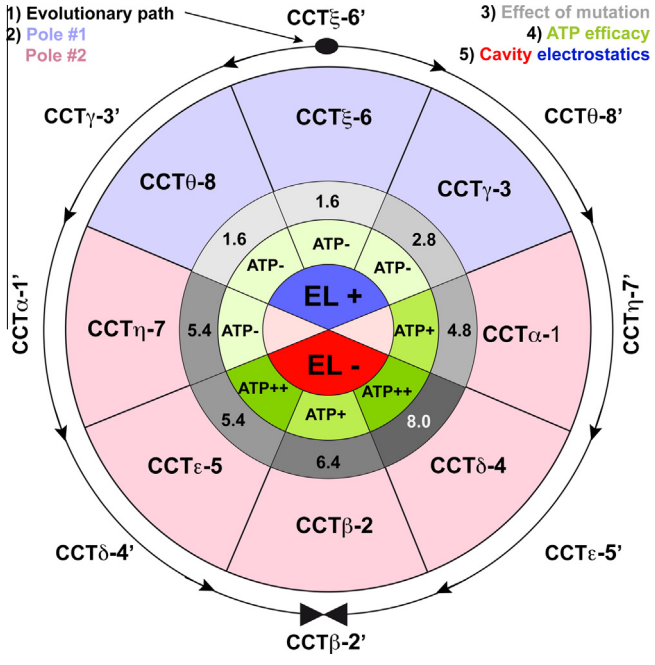

Fig. 6. Polar asymmetry of CCT subunits. A schematic representation of the asymmetry of CCT subunits is shown in layers (from outer to inner; adapted from Ref. [101]). Outer circle (black): possible CCT evolution pathway starting from CCT6 and proceeding through adjacent subunits to CCT2 [101]. Layer 1 (light blue and pink): the two "poles" in the CCT ring [101]. Layer 2 (grey gradient): phenotypic effect of mutations in the ATP binding site of the different subunits (mild to severe effect) [100,103]. Layer 3 (green gradient): the degree of ATP binding and hydrolysis potency in the CCT subunits depicted as ATP++, ATP+ and ATP− [99]. Inner layer: cavity electrostatics [70].

3.3.2. The folding mechanism

Although direct structural information on the thermosome:substrate interaction is lacking, other structural and biochemical studies indicate that the working mechanism of the thermosomes follows the same lines as that of Group I (GroEL), that is, the release of trapped substrate after closure of the chaperonin cavity [73,77].

A more active, mechanical model has been proposed for CCT, in which the conformational changes induced by ATP binding and hydrolysis act on the client protein and promote its correct folding (Fig. 4C) [102,110]. A variation model has also been suggested [11], based on information provided by the crystal structure of the CCT:tubulin complex, in which the sensor loop, after client protein interaction with a specific CCT subunit, would induce ATP binding or vice versa. This second model suggests that this loop could also function as a lever that undergoes conformational change after ATP hydrolysis and helps the substrate through these movements to reach its native conformation. In any case, client protein movements during the CCT functional cycle were confirmed in fluorescent resonance energy transfer (FRET) experiments using fluorophore-labeled actin bound to CCT; these analyses demonstrated a substantial change in donor–acceptor distance after ATP hydrolysis that does not occur in similar experiments with GroEL [111,112]. These data support conformational changes in the substrate after closure of the CCT cavity that are not seen in Group I chaperonins.

3.4. Evolution of Group II chaperonins

Gene duplication is the major driving force for the evolution of Group II chaperonins [7,113]. In Archaea, various processes of gene duplication, loss and conversion have induced the appearance of one, two or three distinct, albeit homologous subunits. Gene duplication is hypothesized to be a result of mutations in the intra-ring subunit domain of one subunit, followed by compensatory changes in another subunit. This situation could have produced a tendency to hetero-oligomer formation, even in the absence of specialized roles for the duplicate subunits.

The same duplication process applies for CCT, which is thought to have appeared early in eukaryotic evolution thanks to "rapid" multiple gene duplication [7]. The driving force in CCT gene duplication could have been its specialization in assistance to the folding of specific substrates, especially its major substrates, the cytoskeletal proteins actin and tubulin [114,115]. This specialization might have occurred through changes in the substrate-binding region of the subunits. Phylogenetic analyses suggest that this duplication process gave rise to CCT4 and CCT5, and more recently to CCT1, CCT2 and CCT7 [7,116], with CCT3, CCT6 and CCT8 as the first CCT subunits to undergo differentiation [101]. Based on the studies described above, an evolutionary pathway suggested for CCT would start in CCT6 and continue circularly through contiguous subunits, to reach CCT2 [101] (Fig. 6). This process first formed the CCT face with the subunits whose ATP binding

and hydrolysis activities are dispensable for the in vivo function of the chaperonin, and generated the most important subunits at the end of the duplication process.

4. Conclusions and future perspectives

Chaperonins are a group of ubiquitous chaperones that share a similar overall structure and function. These molecular machines have a two-ring structure, each of which, driven by ATP binding and hydrolysis, cycles between an open, substrate-receptive state, and a closed state in which a misfolded protein is encapsulated and (re)folding takes place. Despite several similar features, there are important differences among the chaperonins. The most important of these are related to the nature of their substrates, and to the mechanisms of chaperonin-substrate recognition and cavity closure, which are associated to differences in subunit composition and in intra- and inter-ring signaling. Complexity ranges from the simplest GroEL, a homo-heptameric ring that recognizes and encapsulates almost any misfolded protein using a concerted mechanism, to the much more complex CCT, a hetero-octameric ring that recognizes a restricted set of substrates and encapsulates them using a more elaborate mechanism, and which often is assisted by other chaperones. Although a large body of information has been generated on the structure and function of these macromolecular machines, many unanswered questions remain to be addressed to fully understand their complex structural and functional versatility. Structural techniques such as X-ray crystallography and high-resolution cryo-EM, combined with simulations and structural bioinformatics, will provide further answers in the next few years.

Acknowledgements

We apologize for not citing many interesting papers found in the literature owing to space limitations. We thank Dr. G. Montoya for careful reading of the manuscript. We thank Dr. M. Chagoyen for assisting with Fig. 6, and C. Mark for editorial assistance. Our work is funded by grants from the Spanish Ministerio de Ciencia y Competitividad (MINECO) (BFU2013-44202/BMC) and the Madrid Regional Government (S2013/MIT-2807) to JMV, and from the University of Bergen, The Research Council of Norway, the K.G. Jebsen Foundation, and the Meltzer Foundation to LS and AM.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2015.06.019.

References

[1] Hartl, F.U., Bracher, A. and Hayer-Hartl, M. (2011) Molecular chaperones in protein folding and proteostasis. Nature 475, 324–332.
[2] Labbadia, J. and Morimoto, R.I. (2015) The biology of proteostasis in aging and disease. Annu. Rev. Biochem.
[3] Bukau, B., Weissman, J. and Horwich, A. (2006) Molecular chaperones and protein quality control. Cell 125, 443–451.
[4] Yébenes, H., Mesa, P., Muñoz, I.G., Montoya, G. and Valpuesta, J.M. (2011) Chaperonins: two rings for folding. Trends Biochem. Sci. 36, 424–432.
[5] Horwich, A.L. (2013) Chaperonin-mediated protein folding. J. Biol. Chem. 288, 23622–23632.
[6] Mukherjee, K., Conway de Macario, E., Macario, A.J.L. and Brocchieri, L. (2010) Chaperonin genes on the rise: new divergent classes and intense duplication in human and other vertebrate genomes. BMC Evol. Biol. 10, 64.
[7] Archibald, J.M., Logsdon, J.M. and Doolittle, W.F. (2000) Origin and evolution of eukaryotic chaperonins: phylogenetic evidence for ancient duplications in CCT genes. Mol. Biol. Evol. 17, 1456–1466.
[8] Dekker, C., Willison, K.R. and Taylor, W.R. (2011) On the evolutionary origin of the chaperonins. Proteins 79, 1172–1192.
[9] Braig, K., Otwinowski, Z., Hegde, R., Boisvert, D.C., Joachimiak, A., Horwich, A.L. and Sigler, P.B. (1994) The crystal structure of the bacterial chaperonin GroEL at 2.8 Å. Nature 371, 578–586.
[10] Ditzel, L., Löwe, J., Stock, D., Stetter, K.O., Huber, H., Huber, R. and Steinbacher, S. (1998) Crystal structure of the thermosome, the archaeal chaperonin and homolog of CCT. Cell 93, 125–138.
[11] Muñoz, I.G., Yébenes, H., Zhou, M., Mesa, P., Serna, M., Park, A.Y., Bragado-Nilsson, E., Beloso, A., de Cárcer, G., Malumbres, M., Robinson, C.V., Valpuesta, J.M. and Montoya, G. (2011) Crystal structure of the open conformation of the mammalian chaperonin CCT in complex with tubulin. Nat. Struct. Mol. Biol. 18, 14–19.
[12] Clare, D.K., Vasishtan, D., Stagg, S., Quispe, J., Farr, G.W., Topf, M., Horwich, A.L. and Saibil, H.R. (2012) ATP-triggered conformational changes delineate substrate-binding and -folding mechanics of the GroEL chaperonin. Cell 149, 113–123.
[13] Fiaux, J., Bertelsen, E.B., Horwich, A.L. and Wüthrich, K. (2002) NMR analysis of a 900 K GroEL GroES complex. Nature 418, 207–211.
[14] Inobe, T., Takahashi, K., Maki, K., Enoki, S., Kamagata, K., Kadooka, A., Arai, M. and Kuwajima, K. (2008) Asymmetry of the GroEL–GroES complex under physiological conditions as revealed by small-angle X-ray scattering. Biophys. J. 94, 1392–1402.
[15] Skjærven, L., Reuter, N. and Martinez, A. (2011) Dynamics, flexibility and ligand-induced conformational changes in biological macromolecules: a computational approach. Future Med. Chem. 3, 2079–2100.
[16] Xu, Z., Horwich, A.L. and Sigler, P.B. (1997) The crystal structure of the asymmetric GroEL–GroES–(ADP)7 chaperonin complex. Nature 388, 741–750.
[17] Xu, Z. and Sigler, P.B. (1998) GroEL/GroES: structure and function of a two-stroke folding machine. J. Struct. Biol. 124, 129–141.
[18] Bartolucci, C., Lamba, D., Grazulis, S., Manakova, E. and Heumann, H. (2005) Crystal structure of wild-type chaperonin GroEL. J. Mol. Biol. 354, 940–951.
[19] Chaudhry, C., Horwich, A.L., Brunger, A.T. and Adams, P.D. (2004) Exploring the structural dynamics of the *E. coli* chaperonin GroEL using translation-libration-screw crystallographic refinement of intermediate states. J. Mol. Biol. 342, 229–245.
[20] Weissman, J.S., Rye, H.S., Fenton, W.A., Beechem, J.M. and Horwich, A.L. (1996) Characterization of the active intermediate of a GroEL–GroES-mediated protein folding reaction. Cell 84, 481–490.
[21] Horovitz, A. and Willison, K.R. (2005) Allosteric regulation of chaperonins. Curr. Opin. Struct. Biol. 15, 646–651.
[22] Bochkareva, E.S., Lissin, N.M., Flynn, G.C., Rothman, J.E. and Girshovich, a.S. (1992) Positive cooperativity in the functioning of molecular chaperone GroEL. J. Biol. Chem. 267, 6796–6800.
[23] Bochkareva, E. and Girshovich, A. (1994) ATP induces non-identity of two rings in chaperonin GroEL. J. Biol. Chem. 269, 23869–23871.
[24] Gray, T.E. and Fersht, a.R. (1991) Cooperativity in ATP hydrolysis by GroEL is increased by GroES. FEBS Lett. 292, 254–258.
[25] Saibil, H.R., Zheng, D., Roseman, A.M., Hunter, A.S., Watson, G.M.F., Chen, S., auf der Mauer, A., O'Hara, B.P., Wood, S.P., Mann, N.H., Barnett, L.K. and Ellis, R.J. (1993) ATP induces large quaternary rearrangements in a cage-like chaperonin structure. Curr. Biol. 3, 265–273.
[26] Yifrach, O. and Horovitz, A. (1994) Two lines of allosteric communication in the oligomeric chaperonin GroEL are revealed by the single mutation Arg196 Ala. J. Mol. Biol. 243, 397–401.
[27] Yifrach, O. and Horovitz, A. (1995) Nested cooperativity in the ATPase activity of the oligomeric chaperonin GroEL. Biochemistry 34, 5303–5308.
[28] Inobe, T., Makio, T., Takasu-Ishikawa, E., Terada, T.P. and Kuwajima, K. (2001) Nucleotide binding to the chaperonin GroEL: non-cooperative binding of ATP analogs and ADP, and cooperative effect of ATP. BBA-Protein Struct. Mol. 1545, 160–173.
[29] Chaudhry, C., Farr, G.W., Todd, M.J., Rye, H.S., Brunger, A.T., Adams, P.D., Horwich, A.L. and Sigler, P.B. (2003) Role of the gamma-phosphate of ATP in triggering protein folding by GroEL–GroES: function, structure and energetics. EMBO J. 22, 4877–4887.
[30] Monod, J., Wyman, J. and Changeux, J.-P.P. (1965) On the nature of allosteric transitions: a plausible model. J. Mol. Biol. 12, 88–118.
[31] Koshland, D.E., Némethy, G. and Filmer, D. (1966) Comparison of experimental binding data and theoretical models in proteins containing subunits. Biochemistry 5, 365–385.
[32] Cui, Q. and Karplus, M. (2008) Allostery and cooperativity revisited. Protein Sci. 17, 1295–1307.
[33] Roseman, A.M., Chen, S., White, H., Braig, K. and Saibil, H.R. (1996) The chaperonin ATPase cycle: mechanism of allosteric switching and movements of substrate-binding domains in GroEL. Cell 87, 241–251.
[34] Ranson, N.A., Farr, G.W., Roseman, A.M., Gowen, B., Fenton, W.A., Horwich, A.L. and Saibil, H.R. (2001) ATP-bound states of GroEL captured by cryo-electron microscopy. Cell 107, 869–879.
[35] Martin, J., Mayhew, M., Langer, T. and Hartl, F.U. (1993) The reaction cycle of GroEL and GroES in chaperonin-assisted protein folding. Nature 366, 228–233.
[36] Sameshima, T., Iizuka, R., Ueno, T. and Funatsu, T. (2010) Denatured proteins facilitate the formation of the football-shaped GroEL–(GroES)2 complex. Biochem. J. 427, 247–254.

[37] Ye, X. and Lorimer, G.H. (2013) Substrate protein switches GroE chaperonins from asymmetric to symmetric cycling by catalyzing nucleotide exchange. Proc. Natl. Acad. Sci. U.S.A. 110, E4289–E4297.
[38] Ranson, N.A., Clare, D.K., Farr, G.W., Houldershaw, D., Horwich, A.L. and Saibil, H.R. (2006) Allosteric signaling of ATP hydrolysis in GroEL–GroES complexes. Nat. Struct. Mol. Biol. 13, 147–152.
[39] Ma, J., Sigler, P.B., Xu, Z. and Karplus, M. (2000) A dynamic model for the allosteric mechanism of GroEL. J. Mol. Biol. 302, 303–313.
[40] Ma, J. and Karplus, M. (1998) The allosteric mechanism of the chaperonin GroEL: a dynamic analysis. Proc. Natl. Acad. 95, 8502–8507.
[41] Brocchieri, L. and Karlin, S. (2000) Conservation among HSP60 sequences in relation to structure, function, and evolution. Protein Sci. 9, 476–486.
[42] Skjaerven, L., Grant, B., Muga, A., Teigen, K., McCammon, J.A., Reuter, N. and Martinez, A. (2011) Conformational sampling and nucleotide-dependent transitions of the GroEL subunit probed by unbiased molecular dynamics simulations. PLoS Comput. Biol. 7, 14.
[43] Hyeon, C., Lorimer, G.H. and Thirumalai, D. (2006) Dynamics of allosteric transitions in GroEL. Proc. Natl. Acad. Sci. U.S.A. 103, 18939–18944.
[44] Skjaerven, L., Muga, A., Reuter, N. and Martinez, A. (2012) A dynamic model of long-range conformational adaptations triggered by nucleotide binding in GroEL–GroES. Proteins Struct. Funct. Bioinformatics 80, 2333–2346.
[45] Sot, B., von Germar, F., Mantele, W., Valpuesta, J.M., Taneva, S.G., Muga, A. and Mäntele, W. (2005) Ionic interactions at both inter-ring contact sites of GroEL are involved in transmission of the allosteric signal: a time-resolved infrared difference study. Protein Sci. 14, 2267–2274.
[46] Sot, B., Banuelos, S., Valpuesta, J.M., Muga, A. and Bañuelos, S. (2003) GroEL stability and function – contribution of the ionic interactions at the inter-ring contact sites. J. Biol. Chem. 278, 32083–32090.
[47] Lin, Z., Madan, D. and Rye, H.S. (2008) GroEL stimulates protein folding through forced unfolding. Nat. Struct. Mol. Biol. 15, 303–311.
[48] Tyagi, N.K., Fenton, W.A. and Horwich, A.L. (2009) GroEL/GroES cycling: ATP binds to an open ring before substrate protein favoring protein binding and production of the native state. Proc. Natl. Acad. Sci. U.S.A. 106, 20264–20269.
[49] Elad, N., Farr, G.W., Clare, D.K., Orlova, E.V., Horwich, A.L. and Saibil, H.R. (2007) Topologies of a substrate protein bound to the chaperonin GroEL. Mol. Cell 26, 415–426.
[50] Clare, D.K., Bakkes, P.J., van Heerikhuizen, H., van der Vies, S.M. and Saibil, H.R. (2009) Chaperonin complex with a newly folded protein encapsulated in the folding chamber. Nature 457. 107-U113.
[51] Wang, J.M. and Chen, L.L. (2003) Domain motions in GroEL upon binding of an oligopeptide. J. Mol. Biol. 334, 489–499.
[52] Chen, D.-H., Madan, D., Weaver, J., Lin, Z., Schröder, G.F., Chiu, W. and Rye, H.S. (2013) Visualizing GroEL/ES in the act of encapsulating a folding protein. Cell 153, 1354–1365.
[53] Chandrasekhar, G.N., Tilly, K., Woolford, C., Hendrix, R. and Georgopoulos, C. (1986) Purification and properties of the groES morphogenetic protein of *Escherichia coli*. J. Biol. Chem. 261, 12414–12419.
[54] Horwich, A.L., Apetri, A.C. and Fenton, W.A. (2009) The GroEL/GroES cis cavity as a passive anti-aggregation device. FEBS Lett. 583, 2654–2662.
[55] Kanno, R., Koike-Takeshita, A., Yokoyama, K., Taguchi, H. and Mitsuoka, K. (2009) Cryo-EM structure of the native GroEL–GroES complex from *Thermus thermophilus* encapsulating substrate inside the cavity. Structure 17, 287–293.
[56] Tang, Y.-C., Chang, H.-C., Roeben, A., Wischnewski, D., Wischnewski, N., Kerner, M.J., Hartl, F.U. and Hayer-Hartl, M. (2006) Structural features of the GroEL–GroES nano-cage required for rapid folding of encapsulated protein. Cell 125, 903–914.
[57] Burston, S.G., Ranson, N.A. and Clarke, A.R. (1995) The origins and consequences of asymmetry in the chaperonin reaction cycle. J. Mol. Biol. 249, 138–152.
[58] Rye, H.S., Roseman, A.M., Chen, S., Furtak, K., Fenton, W.A., Saibil, H.R. and Horwich, A.L. (1999) GroEL–GroES cycling: ATP and nonnative polypeptide direct alternation of folding-active rings. Cell 97, 325–338.
[59] Grason, J.P., Gresham, J.S. and Lorimer, G.H. (2008) Setting the chaperonin timer: a two-stroke, two-speed, protein machine. Proc. Natl. Acad. Sci. 105, 17339–17344.
[60] Rye, H.S., Burston, S.G., Fenton, W.A., Beechem, J.M., Xu, Z., Sigler, P.B. and Horwich, A.L. (1997) Distinct actions of the cis and trans ATP within the double ring of the chaperonin GroEL. Nature 388, 1287–1292.
[61] Horwich, A.L., Farr, G.W. and Fenton, W.A. (2006) GroEL–GroES-mediated protein folding. Chem. Rev. 106, 1917–1930.
[62] Saibil, H.R. and Ranson, N.A. (2002) The chaperonin folding machine. Trends Biochem. Sci. 27, 627–632.
[63] Llorca, O., Marco, S., Carrascosa, J.L. and Valpuesta, J.M. (1994) The formation of symmetrical GroEL–GroES complexes in the presence of ATP. FEBS Lett. 345, 181–186.
[64] Llorca, O., Marco, S., Carrascosa, J.L. and Valpuesta, J.M. (1997) Symmetric GroEL–GroES complexes can contain substrate simultaneously in both GroEL rings. FEBS Lett. 405, 195–199.
[65] Azem, A., Kessel, M. and Goloubinoff, P. (1994) Characterization of a functional GroEL14 (GroES7) 2 chaperonin hetero-oligomer. Science 265, 653–656.
[66] Schmidt, M., Rutkat, K., Rachel, R., Pfeifer, G., Jaenicke, R., Viitanen, P., Lorimer, G. and Buchner, J. (1994) Symmetric complexes of GroE chaperonins as part of the functional cycle. Science 265, 656–659.
[67] Fei, X., Ye, X., LaRonde, N.A. and Lorimer, G.H. (2014) Formation and structures of GroEL:GroES2 chaperonin footballs, the protein-folding functional form. Proc. Natl. Acad. Sci. 111, 2–7.
[68] Bigotti, M.G. and Clarke, A.R. (2008) Chaperonins: the hunt for the Group II mechanism. Arch. Biochem. Biophys. 474, 331–339.
[69] Valpuesta, J.M., Martín-Benito, J., Gómez-Puertas, P., Carrascosa, J.L. and Willison, K.R. (2002) Structure and function of a protein folding machine: the eukaryotic cytosolic chaperonin CCT. FEBS Lett. 529, 11–16.
[70] Kalisman, N., Adams, C.M. and Levitt, M. (2012) Subunit order of eukaryotic TRiC/CCT chaperonin by cross-linking, mass spectrometry, and combinatorial homology modeling. Proc. Natl. Acad. Sci. U.S.A. 109, 2884–2889.
[71] Leitner, A., Joachimiak, L.A., Bracher, A., Mönkemeyer, L., Walzthoeni, T., Chen, B., Pechmann, S., Holmes, S., Cong, Y., Ma, B., Ludtke, S., Chiu, W., Hartl, F.U., Aebersold, R. and Frydman, J. (2012) The molecular architecture of the eukaryotic chaperonin TRiC/CCT. Structure 20, 814–825.
[72] Cong, Y., Baker, M.L., Jakana, J., Woolford, D., Miller, E.J., Reissmann, S., Kumar, R.N., Redding-Johanson, A.M., Batth, T.S., Mukhopadhyay, A., Ludtke, S.J., Frydman, J. and Chiu, W. (2010) 4.0-A resolution cryo-EM structure of the mammalian chaperonin TRiC/CCT reveals its unique subunit arrangement. Proc. Natl. Acad. Sci. U.S.A. 107, 4967–4972.
[73] Pereira, J.H., Ralston, C.Y., Douglas, N.R., Meyer, D., Knee, K.M., Goulet, D.R., King, J.A., Frydman, J. and Adams, P.D. (2010) Crystal structures of a group II chaperonin reveal the open and closed states associated with the protein folding cycle. J. Biol. Chem. 285, 27958–27966.
[74] Huo, Y., Hu, Z., Zhang, K., Wang, L., Zhai, Y., Zhou, Q., Lander, G., Zhu, J., He, Y., Pang, X., Xu, W., Bartlam, M., Dong, Z. and Sun, F. (2010) Crystal structure of group II chaperonin in the open state. Structure 18, 1270–1279.
[75] Zhang, J., Ma, B., Dimaio, F., Douglas, N.R., Joachimiak, L.a., Baker, D., Frydman, J., Levitt, M. and Chiu, W. (2011) Cryo-EM structure of a group II chaperonin in the prehydrolysis ATP-bound state leading to lid closure. Structure 19, 633–639.
[76] 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. and Chiu, W. (2010) Mechanism of folding chamber closure in a group II chaperonin. Nature 463, 379–383.
[77] Douglas, N.R., Reissmann, S., Zhang, J., Chen, B., Jakana, J., Kumar, R., Chiu, W. and Frydman, J. (2011) Dual action of ATP hydrolysis couples lid closure to substrate release into the group II chaperonin chamber. Cell 144, 240–252.
[78] Dekker, C., Roe, S.M., McCormack, E.A., Beuron, F., Pearl, L.H. and Willison, K.R. (2011) The crystal structure of yeast CCT reveals intrinsic asymmetry of eukaryotic cytosolic chaperonins. EMBO J. 30, 3078–3090.
[79] Lin, P. and Sherman, F. (1997) The unique hetero-oligomeric nature of the subunits in the catalytic cooperativity of the yeast Cct chaperonin complex. Proc. Natl. Acad. Sci. 94, 10780–10785.
[80] Kafri, G. and Horovitz, A. (2003) Transient kinetic analysis of ATP-induced allosteric transitions in the eukaryotic chaperonin containing TCP-1. J. Mol. Biol. 326, 981–987.
[81] Rivenzon-Segal, D., Wolf, S.G., Shimon, L., Willison, K.R. and Horovitz, A. (2005) Sequential ATP-induced allosteric transitions of the cytoplasmic chaperonin containing TCP-1 revealed by EM analysis. Nat. Struct. Mol. Biol. 12, 233–237.
[82] Shimon, L., Hynes, G.M., McCormack, E.A., Willison, K.R. and Horovitz, A. (2008) ATP-induced allostery in the eukaryotic chaperonin CCT is abolished by the mutation G345D in CCT4 that renders yeast temperature-sensitive for growth. J. Mol. Biol. 377, 469–477.
[83] Valpuesta, J.M., Carrascosa, J.L. and Willison, K.R. (2008) Structure and function of the cytosolic chaperonin CCTProtein Folding Handbook, pp. 725–755, Wiley-VCH Verlag GmbH, Weinheim, Germany.
[84] Kusmierczyk, A.R. and Martin, J. (2003) Nested cooperativity and salt dependence of the ATPase activity of the archaeal chaperonin Mm-cpn. FEBS Lett. 547, 201–204.
[85] Bigotti, M.G. and Clarke, A.R. (2005) Cooperativity in the thermosome. J. Mol. Biol. 348, 13–26.
[86] Shomura, Y., Yoshida, T., Iizuka, R., Maruyama, T., Yohda, M. and Miki, K. (2004) Crystal structures of the group II chaperonin from Thermococcus strain KS-1: steric hindrance by the substituted amino acid, and inter-subunit rearrangement between two crystal forms. J. Mol. Biol. 335, 1265–1278.
[87] Reissmann, S., Parnot, C., Booth, C.R., Chiu, W. and Frydman, J. (2007) Essential function of the built-in lid in the allosteric regulation of eukaryotic and archaeal chaperonins. Nat. Struct. Mol. Biol. 14, 432–440.
[88] Kanzaki, T., Iizuka, R., Takahashi, K., Maki, K., Masuda, R., Sahlan, M., Yebenes, H., Valpuesta, J.M., Oka, T., Furutani, M., Ishii, N., Kuwajima, K. and Yohda, M. (2008) Sequential action of ATP-dependent subunit conformational change and interaction between helical protrusions in the closure of the built-in lid of Group II chaperonins. J. Biol. Chem. 283, 34773–34784.
[89] Pereira, J.H., Ralston, C.Y., Douglas, N.R., Kumar, R., Lopez, T., McAndrew, R.P., Knee, K.M., King, J.A., Frydman, J. and Adams, P.D. (2012) Mechanism of nucleotide sensing in group II chaperonins. EMBO J. 31, 731–740.
[90] Spiess, C., Miller, E.J., McClellan, A.J. and Frydman, J. (2006) Identification of the TRiC/CCT substrate binding sites uncovers the function of subunit diversity in eukaryotic chaperonins. Mol. Cell 24, 25–37.
[91] Ho, Y., Gruhler, A., Heilbut, A., Bader, G.D., Moore, L., Adams, S.-L., Millar, A., Taylor, P., Bennett, K., Boutilier, K., Yang, L., Wolting, C., Donaldson, I., Schandorff, S., Shewnarane, J., Vo, M., Taggart, J., Goudreault, M., Muskat, B., Alfarano, C., Dewar, D., Lin, Z., Michalickova, K., Willems, A.R., Sassi, H.,

Nielsen, K.H., Rasmussen, K.J., Andersen, J.R., Johansen, L.E., Hansen, L.H., Jespersen, H., Podtelejnikov, A., Nielsen, E., Crawford, J., Poulsen, V., Sørensen, B.D., Matthiesen, J., Hendrickson, R.C., Gleeson, F., Pawson, T., Moran, M.F., Durocher, D., Mann, M., Hogue, C.W.V., Figeys, D. and Tyers, M. () Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. Nature 415, 180–183.

[92] Plimpton, R.L., Cuéllar, J., Lai, C.W.J., Aoba, T., Makaju, A., Franklin, S., Mathis, A.D., Prince, J.T., Carrascosa, J.L., Valpuesta, J.M. and Willardson, B.M. (2015) Structures of the Gβ-CCT and PhLP1-Gβ-CCT complexes reveal a mechanism for G-protein β-subunit folding and Gβγ dimer assembly. Proc. Natl. Acad. Sci. U.S.A. 112, 2413–2418.

[93] Martín-Benito, J., Grantham, J., Boskovic, J., Brackley, K.I., Carrascosa, J.L., Willison, K.R. and Valpuesta, J.M. (2007) The inter-ring arrangement of the cytosolic chaperonin CCT. EMBO Rep. 8, 252–257.

[94] Feldman, D.E., Thulasiraman, V., Ferreyra, R.G. and Frydman, J. (1999) Formation of the VHL–Elongin BC tumor suppressor complex is mediated by the chaperonin TRiC. Mol. Cell 4, 1051–1061.

[95] Cuéllar, J., Martín-Benito, J., Scheres, S.H.W., Sousa, R., Moro, F., López-Viñas, E., Gómez-Puertas, P., Muga, A., Carrascosa, J.L. and Valpuesta, J.M. (2008) The structure of CCT-Hsc70 NBD suggests a mechanism for Hsp70 delivery of substrates to the chaperonin. Nat. Struct. Mol. Biol. 15, 858–864.

[96] Martín-Benito, J., Bertrand, S., Hu, T., Ludtke, P.J., McLaughlin, J.N., Willardson, B.M., Carrascosa, J.L. and Valpuesta, J.M. (2004) Structure of the complex between the cytosolic chaperonin CCT and phosducin-like protein. Proc. Natl. Acad. Sci. U.S.A. 101, 17410–17415.

[97] Stirling, P.C., Cuéllar, J., Alfaro, G.A., El Khadali, F., Beh, C.T., Valpuesta, J.M., Melki, R. and Leroux, M.R. (2006) PhLP3 modulates CCT-mediated actin and tubulin folding via ternary complexes with substrates. J. Biol. Chem. 281, 7012–7021.

[98] Lopez-Fanarraga, M., Avila, J., Guasch, A., Coll, M. and Zabala, J.C. (2001) Review: postchaperonin tubulin folding cofactors and their role in microtubule dynamics. J. Struct. Biol. 135, 219–229.

[99] Reissmann, S., Joachimiak, L.A., Chen, B., Meyer, A.S., Nguyen, A. and Frydman, J. (2012) A gradient of ATP affinities generates an asymmetric power stroke driving the chaperonin TRIC/CCT folding cycle. Cell Rep. 2, 866–877.

[100] Kalisman, N., Schröder, G.F. and Levitt, M. (2013) The crystal structures of the eukaryotic chaperonin CCT reveal its functional partitioning. Structure 21, 540–549.

[101] Chagoyen, M., Carrascosa, J.L., Pazos, F. and Valpuesta, J.M. (2014) Molecular determinants of the ATP hydrolysis asymmetry of the CCT chaperonin complex. Proteins 82, 703–707.

[102] Llorca, O., Martín-Benito, J., Grantham, J., Ritco-Vonsovici, M., Willison, K.R., Carrascosa, J.L. and Valpuesta, J.M. (2001) The "sequential allosteric ring" mechanism in the eukaryotic chaperonin-assisted folding of actin and tubulin. EMBO J. 20, 4065–4075.

[103] Amit, M., Weisberg, S.J., Nadler-Holly, M., McCormack, E.A., Feldmesser, E., Kaganovich, D., Willison, K.R. and Horovitz, A. (2010) Equivalent mutations in the eight subunits of the chaperonin CCT produce dramatically different cellular and gene expression phenotypes. J. Mol. Biol. 401, 532–543.

[104] Hirtreiter, A.M., Calloni, G., Forner, F., Scheibe, B., Puype, M., Vandekerckhove, J., Mann, M., Hartl, F.U. and Hayer-Hartl, M. (2009) Differential substrate specificity of group I and group II chaperonins in the archaeon *Methanosarcina mazei*. Mol. Microbiol. 74, 1152–1168.

[105] Gómez-Puertas, P., Martín-Benito, J., Carrascosa, J.L., Willison, K.R. and Valpuesta, J.M. (2004) The substrate recognition mechanisms in chaperonins. J. Mol. Recognit. 17, 85–94.

[106] Kasembeli, M., Lau, W.C.Y., Roh, S.H., Eckols, T.K., Frydman, J., Chiu, W. and Tweardy, D.J. (2014) Modulation of STAT3 folding and function by TRiC/CCT chaperonin. PLoS Biol. 12, e1001844.

[107] Suzuki, M., Ueno, T., Iizuka, R., Miura, T., Zako, T., Akahori, R., Miyake, T., Shimamoto, N., Aoki, M., Tanii, T., Ohdomari, I. and Funatsu, T. (2008) Effect of the C-terminal truncation on the functional cycle of chaperonin GroEL: implication that the C-terminal region facilitates the transition from the folding-arrested to the folding-competent state. J. Biol. Chem. 283, 23931–23939.

[108] Bergeron, L.M., Shis, D.L., Gomez, L. and Clark, D.S. (2009) Small molecule inhibition of a Group II chaperonin: pinpointing a loop region within the equatorial domain as necessary for protein refolding. Arch. Biochem. Biophys. 481, 45–51.

[109] Herzog, F., Kahraman, A., Boehringer, D., Mak, R., Bracher, A., Walzthoeni, T., Leitner, A., Beck, M., Hartl, F.-U., Ban, N., Malmström, L. and Aebersold, R. (2012) Structural probing of a protein phosphatase 2A network by chemical cross-linking and mass spectrometry. Science 337, 1348–1352.

[110] Stuart, S.F., Leatherbarrow, R.J. and Willison, K.R. (2011) A two-step mechanism for the folding of actin by the yeast cytosolic chaperonin. J. Biol. Chem. 286, 178–184.

[111] Villebeck, L., Moparthi, S.B., Lindgren, M., Hammarström, P. and Jonsson, B.-H. (2007) Domain-specific chaperone-induced expansion is required for beta-actin folding: a comparison of beta-actin conformations upon interactions with GroEL and tail-less complex polypeptide 1 ring complex (TRiC). Biochemistry 46, 12639–12647.

[112] Villebeck, L., Persson, M., Luan, S.-L., Hammarström, P., Lindgren, M. and Jonsson, B.-H. (2007) Conformational rearrangements of tail-less complex polypeptide 1 (TCP-1) ring complex (TRiC)-bound actin. Biochemistry 46, 5083–5093.

[113] Archibald, J.M. and Roger, A.J. (2002) Gene duplication and gene conversion shape the evolution of archaeal chaperonins. J. Mol. Biol. 316, 1041–1050.

[114] K.R. Willison, H. Kubota, The structure, function, and genetics of the chaperonin containing TCP-1 (CCT) in eukaryotic cytosol, in: The biology of heat shock proteins and molecular chaperones, Cold Spring Harbor Laboratory Press, New York, NY, Cold Spring Harbor, NY, 299–312, 1994.

[115] K.R. Willison, A.L. Horwich, Structure and function of chaperonins in archaebacteria and eukaryotic cytosol, in: The Chaperonins, 107–136, 1996.

[116] Fares, M.A. and Wolfe, K.H. (2003) Positive selection and subfunctionalization of duplicated CCT chaperonin subunits. Mol. Biol. Evol. 20, 1588–1597.