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Allosteric regulation of chaperonins

Amnon Horovitz[1] and Keith R Willison[2]

Chaperonins are molecular machines that facilitate protein folding by undergoing energy (ATP)-dependent movements that are coordinated in time and space by complex allosteric regulation. Recently, progress has been made in describing the various functional (allosteric) states of these machines, the pathways by which they interconvert, and the coupling between allosteric transitions and protein folding reactions. However, various mechanistic issues remain to be resolved.

Addresses
[1] Department of Structural Biology, Weizmann Institute of Science, Rehovot 76100, Israel
[2] Institute of Cancer Research, Cancer Research UK Centre for Cell and Molecular Biology, Chester Beatty Laboratories, London SW3 6JB, UK

Corresponding author: Horovitz, Amnon (amnon.horovitz@weizmann.ac.il)

Current Opinion in Structural Biology 2005, **15**:646–651

This review comes from a themed issue on
Catalysis and regulation
Edited by William N Hunter and Ylva Lindqvist

Available online 24th October 2005

0959-440X/$ – see front matter

DOI 10.1016/j.sbi.2005.10.001

Introduction

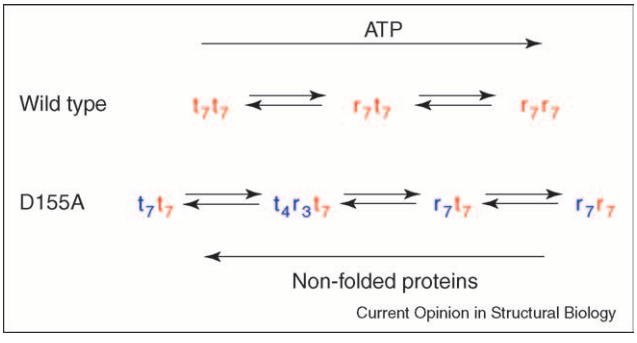
The classical Monod-Wyman-Changeux (MWC) [1] and Koshland-Némethy-Filmer (KNF) [2] models of cooperativity were originally formulated in the 1960s with metabolic regulation of enzyme activity in mind. More recently, it has become apparent that allosteric theory is also required for describing the workings of various biomolecular machines. The Webster's dictionary definition of a machine is: ''an assemblage of parts that are usually solid bodies (but include in some cases fluid bodies or electricity in conductors) and that transmit forces, motion and energy one to another in some predetermined manner and to some desired end''. Chaperonins are molecular machines that facilitate protein folding by undergoing energy (ATP)-dependent rigid-body movements [3,4] that are coordinated in time and space by complex allosteric regulation [5–7]. They are made up of two oligomeric rings, stacked back-to-back, with a cavity at each end that provides a protective environment for protein folding. Chaperonins can be divided into two groups: group I, found in eubacteria, mitochondria and chloroplasts [6,7]; and group II, found in archaea and the eukaryotic cytosol [8–10]. Group I chaperonins consist of two identical (as in GroEL from *Escherichia coli*) or non-identical (as in chloroplast chaperonins) homo-oligomeric rings [6,7]. Group II chaperonins consist of two identical eight- or nine-membered hetero-oligomeric rings comprising two types of subunits in the case of the *Thermoplasma acidophilum* thermosome or eight different subunits in the case of the cytoplasmic eukaryotic chaperonin containing TCP-1 (CCT) [8–10]. Crystal structures of GroEL [11] and the thermosome from *T. acidophilum* [12] indicate that group I and II chaperonins share a similar domain arrangement. Each subunit consists of three domains: an equatorial domain that contains an ATP-binding site; an apical domain that forms the opening of the central cavity and binds non-folded polypeptide substrates; and an intermediate domain that connects the apical and equatorial domains. Group I chaperonins function in conjunction with a heptameric ring-shaped co-chaperonin, such as GroES in *E. coli*, that caps the cavity of the so-called *cis* ring in the **R** state [13], thereby triggering the dissociation of apical-domain-bound protein substrates into the cavity. By contrast, group II chaperonins operate without a GroES homologue, whose function appears to be mimicked [14] by an extra sequence located at the tip of the apical domain — the 'helical protrusion' [8,12].

Allosteric regulation is responsible for the transitions between different functional states of proteins (or other macromolecules) in response to changes in environmental conditions. It is often achieved via changes in the conformation of multimeric proteins induced by ligand binding [15]. Such a mechanism can also lead to the repeated cycling between different functional states that is characteristic of molecular machines. Hence, a preliminary understanding of how chaperonins function as machines requires knowing their main allosteric states and associated functional properties. A deeper question concerns the nature of the transitions between the relatively stable different allosteric states. In other words, do populated kinetic intermediates exist? Are there single or parallel pathways between states? Such questions are often ignored (and might indeed be of little importance) when dealing with allosteric regulation in the context of metabolic control (e.g. oxygen uptake and release by haemoglobin), but they are of considerable potential significance for molecular machines because their efficiency may be path dependent. Finally, one would like to understand the mechanism of coupling between conformational changes in chaperonins and the folding process in structural and energetic terms. Progress with regard to our understanding of the above issues will be reviewed in what follows.

Intra-ring and inter-ring allostery

Steady-state kinetic measurements of initial rates of ATP hydrolysis by GroEL at different concentrations of ATP showed that GroEL undergoes two ATP-induced allosteric transitions: one with a midpoint at relatively low ATP concentrations and the second with a midpoint at higher concentrations of ATP [16,17]. Each of the allosteric transitions is reflected in intra-ring positive cooperativity in ATP binding and hydrolysis by GroEL, with respect to ATP [18,19] and K^+ [20]. The higher ATP concentration required to effect the second allosteric transition reflects inter-ring negative cooperativity in ATP binding. A nested allosteric model (Figure 1) of cooperativity in ATP binding by GroEL that accounts for these findings was put forward [16,17], in which, in accordance with the MWC representation [1], each ring is in equilibrium between two states: a tense ($\mathbf{t}_7$, **T**) state, with low affinity for ATP and high affinity for non-folded protein substrates; and a relaxed ($\mathbf{r}_7$, **R**) state, with high affinity for ATP and low affinity for non-folded protein substrates [21,22]. The **T** and **R** states are, therefore, protein substrate acceptor and release states, respectively. In the presence of increasing concentrations of ATP, the GroEL double-ring switches in a sequential manner from the **TT** state (both rings are in the **T** state) via the **TR** state to the **RR** state, in accordance with the KNF model [2]. Hence, MWC-type allosteric interactions that lead to intra-ring positive cooperativity in ATP binding by GroEL are nested in KNF-type allosteric interactions that lead to inter-ring negative cooperativity in ATP binding. Plots of the observed rate constant of the $\mathbf{T} \rightarrow \mathbf{R}$ transition as a function of ATP concentration for double-ring GroEL variants (in which the mutations F44W [23], Y485W [24] or R231W [25] were introduced to facilitate the following of ATP-induced conformational changes by monitoring time-resolved changes in fluorescence) have also been found to be bi-sigmoidal. By contrast, a plot of initial rates of ATP hydrolysis by a single-ring version of GroEL (SR1) at different concentrations of ATP was found to be mono-sigmoidal [26], indicating that it undergoes only one allosteric transition, as predicted by the nested model.

Figure 1



Scheme showing the different allosteric states of wild-type GroEL and the D155A mutant. In this scheme, **t** and **r** represent the conformation of a subunit in the **T** and **R** states, respectively, and $\mathbf{t}_n\mathbf{r}_{7-n}$ indicates a ring with n adjacent subunits in the **t** state and 7–n adjacent subunits in the **r** state. In the absence of ligands, GroEL is predominantly in the relatively symmetric $\mathbf{t}_7\mathbf{t}_7$ state. The break in symmetry between rings in the D155A mutant is indicated by the blue and red colours. In the presence of ATP, the equilibrium of the ATP-bound ring is shifted toward the $\mathbf{r}_7$ state in the case of wild-type GroEL, and the $\mathbf{t}_4\mathbf{r}_3$ and $\mathbf{r}_7$ states in the case of the D155A mutant. A further shift in the equilibrium toward the $\mathbf{r}_7\mathbf{r}_7$ state takes place at higher ATP concentrations. Non-folded protein substrates shift the equilibrium in the opposite direction.

Considerable variation has been found in the intra-ring allosteric properties of chaperonins. Homo-oligomeric GroEL displays positive cooperativity in ATP hydrolysis, with respect to ATP, whereas in the hetero-oligomeric archaeal chaperonin from *Methanococcus maripaludis* (Mm-cpn60) [27] and CCT [28], such intra-ring cooperativity is relatively weak and in the *T. acidophilum* thermosome, it appears to be absent [29]. A possible explanation for these differences is that positive cooperativity owing to the ATP-induced intra-ring allosteric transitions of group II chaperonins is masked by apparent negative cooperativity in ATP binding and/or hydrolysis stemming from the subunit heterogeneity of these chaperonins [28]. In addition, the intra-ring allosteric transitions of CCT are sequential [30•], whereas those of GroEL appear to be concerted (see below).

By contrast, inter-ring negative cooperativity in ATP binding appears to be a universally conserved property of all chaperonins, as it has been observed also in the case of the group II chaperonins CCT [28,31], Mm-cpn60 [27] and the thermosome [29]. This is despite the fact that the role of inter-ring allostery in GroEL is associated with the function of GroES, whereas group II chaperonins appear to operate without a GroES homologue. In the case of the GroE system, ATP binding to the distal (*trans*) ring sends an allosteric signal that triggers GroES dissociation from the *cis* ring, thereby releasing polypeptide substrates from the GroES-bound *cis* ring into solution [32]. The rate of GroES dissociation is increased upon stabilising the distal ring's **T** state by mutation [33] or binding of non-folded protein substrates [34]. This effect is mirrored by the observation that GroES binding to the *cis* ring decreases cooperativity in ATP binding by the *trans* ring [35], thereby promoting the release of protein substrates from the *trans* ring [36]. A conundrum is posed by the observation that GroES dissociation from the *cis* ring depends on ATP binding to the *trans* ring (which is expected to stabilise it in an **R**-like state), but is accelerated upon stabilisation of the *trans* ring in a **T**-like state. Although the mechanism of inter-ring signalling remains unknown, a transient kinetic phase associated with this process has recently been identified using the F44W [37] but not Y485W [38] probe. Interestingly, the value of the rate constant corresponding to this phase is higher in mutants with increased intra-ring positive cooperativity, in agree-

ment with simulations that indicated coupling between inter-ring and intra-ring allostery [39]. Evidence of coupling between inter-ring and intra-ring allostery is also provided by the finding that conversion of the out-of-register alignment of contacts between subunits of opposing rings seen in wild-type GroEL to an in-register one by the mutation E461K causes intra-ring cooperativity to be abolished [40•]. Inter-ring coupling in GroEL is also reflected in a higher Arrhenius activation enthalpy of ATP hydrolysis for wild-type GroEL compared with SR1 [41] and in a decrease in ATPase activity at high ATP concentrations when ADP must dissociate from one ring before the other ring can hydrolyse. This decrease is not observed in the R13G, A126V [42] and E257A (A Horovitz, unpublished) GroEL mutants or in CCT [28]. Future construction of single-ring versions of group II chaperonins is likely to shed light on the role of inter-ring communication in their reaction cycles, which at present remains unknown.

Structural analysis of allosteric states

The structure of the **TT** state has been solved at high resolution in the case of the R13G/A126V GroEL mutant [11] and, more recently, at a resolution of 6 Å by electron cryo-microscopy (cryo-EM) for wild-type GroEL [43•]. In the case of group II chaperonins, the corresponding structures, referred to as the 'open' state, have been visualised using EM (e.g. [44]) but not in crystals [12], probably owing to crystal packing and buffer conditions [45]. Low-resolution cryo-EM studies of R197A GroEL [46] and the thermosome [47] have revealed structures that correspond to the **TT**, **TR** and **RR** states. The notation of **T** and **R** refers, however, to all the various low- and high-affinity states for ATP of an individual ring [17] that can be distinguished by higher resolution structural studies. For example, cryo-EM studies of unliganded GroEL have revealed a small but distinct asymmetry between rings [3,48•], suggesting that a more appropriate notation for this state might be **TT**′. In addition, recent work has shown that the **TT** state undergoes structural changes upon polypeptide binding [48•,49]. X-ray analysis of a GroEL–peptide$_{14}$ complex showed that the apical domains rotate clockwise within one GroEL ring [49], whereas ATP-induced apical domain rotation is counter-clockwise [3,46]. By contrast, cryo-EM analysis of the structure of GroEL bound to a single monomer of glutamine synthetase at 13 Å resolution showed that the apical domains of both the substrate-bound and substrate-free rings undergo counter-clockwise rotations (although not as dramatic as those documented for the ATP-induced structural changes) [48•].

There are also unresolved issues with regard to the various **R** states, designated as 'closed' in the case of type II chaperonins. For example, there is controversy concerning whether ATP binding is sufficient [4,14] or whether hydrolysis is also required [50] to reach the 'closed' state. The crystal structure of the thermosome in complex with ADP•AlF_3 [12] shows that D390 (equivalent to D398 in GroEL) is ligated to AlF_3. The D398A mutant of GroEL was reported to have *2%* wild-type ATPase activity [32] and simulations have suggested that the **T** → **R** transition in GroEL begins with a downward motion of helix M that brings D398 into the coordination sphere of the nucleotide-bound Mg^{2+} [51]. Hence, it is puzzling that the **T** state of GroEL is catalytically more active than its **R** state [22], suggesting that perhaps different sets of residues in combination with D398 are involved in catalysis by the two states. The contribution of K^+ to catalysis of ATP hydrolysis by GroEL has recently become somewhat clearer, as a result of the refined crystal structure of the R13G/A126V mutant in what should be the **RR** state (but may not be owing to crystal packing forces and the mutations). The structure shows, for the first time, the ligation of K^+ to ATP [52]. Finally, also not fully understood is the observation that crystal structures of the **TR** state in complex with GroES and ADP [13] or ADP•AlF_3 [53•] appear to be similar, despite significant differences in stability and function between the two types of complexes. Interestingly, a crystal structure of the GroEL–GroES complex from *Thermus thermophilus* with bound substrates shows unexpected asymmetry in the *cis* cavity [54•], thus reinforcing the idea that intra-ring symmetry in chaperonins is not always maintained [30•,55•].

Pathways of allosteric transitions

Several lines of evidence indicate that the allosteric transitions of GroEL are concerted ([51,56]; G Lorimer, personal communication). Interestingly, mutation D155A in GroEL, which breaks an intrasubunit salt bridge with R395, converts its intra-ring allosteric transition from concerted to sequential [55•] (Figure 1), thereby demonstrating that cooperativity in this system is due to coupled tertiary conformational changes [51]. By contrast, genetic [57] and EM [30•] evidence indicates that the intra-ring allosteric transitions of CCT are sequential. ϕ-value analysis [58] of the ATP-induced allosteric transitions in GroEL has shown that the R197–E386 intersubunit salt bridge is broken in the transition state of the **T** → **R** switch [59], which takes place via at least two parallel pathways [60] (although this has been contested [61]). Evidence of dual pathways is also provided by the bi-sigmoidal dependence of the rate of the **T** → **R** switch on ATP concentration observed for certain single-ring versions of GroEL [25,37]. Indication that the R197–E386 salt bridge is important in the allosteric mechanism of GroEL has also come from simulations [51]. It has been suggested that, following the breaking of the R197–E386 salt bridge, a new intersubunit salt link is formed between E386 and K80 [3]. Such switching of salt bridges has been observed in the case of other allosteric proteins [15]. The dramatic effects of the D155A mutation [55•] suggest, however, that the allosteric mechanism of GroEL is more

complex and involves other interactions. This complexity is also manifested in the observation that single amino acid changes at diverse positions throughout SR1 reduce GroES binding without affecting nucleotide binding, thereby restoring chaperone activity [62].

Allostery and folding

It has been shown that the extent of cooperativity in GroEL, with respect to ATP binding, can affect the rate of substrate folding, probably because the rate of the **T** → **R** transition is rate limiting [63]. It is not known, however, whether efficient GroE-assisted folding is dependent on the concerted nature of the **T** → **R** transition. Concerted ATP-induced conformational changes in GroEL may lead to the simultaneous release of different parts of the protein substrate, thereby increasing folding rates and/or yields. By contrast, ATP-induced sequential conformational changes in CCT may facilitate sequential release and folding of individual domains of multidomain protein substrates, thereby mimicking co-translational folding (thought to be more common in eukaryotes [64]). It has been suggested that the ATP-induced **T** → **R** transition can lead to forced unfolding (or stretching), thereby providing misfolded proteins further opportunity to fold [65]. Evidence in support of this mechanism was not found in the case of malate dehydrogenase [66] and the evidence supporting this proposal in the case of Rubisco has recently been questioned [67]. The observation that GroEL-bound Rubisco becomes more compact following ATP- and GroES-induced release [68] also seems to be inconsistent with the stretching model. Although this model is now in doubt, the notion that GroEL carries out work on bound substrates is suggested by fluorescence resonance energy transfer (FRET) data [69•]. Horwich and co-workers showed that, in the absence of polypeptide substrate, the rates and extents of the GroES/ADP- and GroES/ATP-induced conformational changes are similar. However, in the presence of polypeptide substrate, a similar rate and extent of FRET was observed in the presence of GroES/ATP but not GroES/ADP [69•]. Hence, polypeptide binding [69•] or mutations [63] that retard the allosteric transitions of GroEL (by stabilising its **T** state) lead to decreased folding. In the case of polypeptide binding, this effect can be reversed by GroES/ATP but not GroES/ADP, thus helping to explain why GroEL in complex with GroES/ADP is not folding active.

Conclusions

The identity of *in vivo* substrates [54•] and substrate recognition mechanisms [70,71] are two still unresolved issues that have not been discussed in this review (see Update). Another open issue concerns the possible effects of chaperonins on folding pathways. For example, are transition states of folding in bulk solution the same as those in the cavity of chaperonins under conditions of confinement [72]? Spectroscopic (e.g. FRET) single-molecule techniques are likely to contribute to our understanding of such questions, whereas mechanical single-molecule techniques may shed light on aspects of chaperonin function such as forced unfolding. Further progress in understanding the allosteric mechanisms of chaperonins will also depend on the availability of crystal structures of CCT and wild-type GroEL, and high-resolution EM structures that represent different conformational states in solution.

Update

Recently, there has been further progress in identifying obligate *in vivo* substrates of GroEL [73•] and in characterising the conformational states of a chaperonin-bound folding intermediate of a model substrate [74•].

Acknowledgements

This work was supported by The Israel Science Foundation. AH is an incumbent of the Carl and Dorothy Bennett Professorial Chair in Biochemistry. KRW is supported by Cancer Research UK. This review was written whilst AH was a Visiting Professor at the Chemical Biology Centre, Imperial College, London.

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