Journal of Structural Biology **135,** 95–103 (2001)
doi:10.1006/jsbi.2001.4387, available online at http://www.idealibrary.com on IDEAL®

Review: A Structural View of the GroE Chaperone Cycle

Holger Grallert and Johannes Buchner[1]

Institut für Organische Chemie und Biochemie, Technische Universität München, 85747 Garching, Germany

Received March 1, 2001, and in revised form May 23, 2001

The GroE chaperone system consists of two ring-shaped oligomeric components whose association creates different functional states. The most remarkable property of the GroE system is the ability to fold proteins under conditions where spontaneous folding cannot occur. To achieve this, a fully functional system consisting of GroEL, the cochaperone GroES, and ATP is necessary. Driven by ATP binding and hydrolysis, this system cycles through different conformational stages, which allow binding, folding, and release of substrate proteins. Some aspects of the ATP-driven reaction cycle are still under debate. One of these open questions is the importance of so-called "football" complexes consisting of GroEL and two bound GroES rings. Here, we summarize the evidence for the functional relevance of these complexes and their involvement in the efficient folding of substrate proteins.

INTRODUCTION

GroEL is an oligomeric complex of 14 identical 57-kDa subunits, arranged in 2 seven-membered rings sticking back to back. The crystal structure of GroEL determined at a resolution of 2.8 Å shows a hollow, cylindrical complex 135 Å in diameter and with a height of 145 Å (Braig *et al.*, 1994). The openings at each end of the cylinder form the entrance to the central cavity with a diameter of 45 Å. Nonnative polypeptides are bound at these openings as shown by electron microscopy (Langer *et al.*, 1992; Braig *et al.*, 1993; Chen *et al.*, 1994; Ishii *et al.*, 1994).

The GroEL monomer consists of 547 amino acids arranged in three distinct domains (Fig. 1). The equatorial domain is responsible for the inter- and intra-ring interactions of the protein complex. Furthermore, this domain contains the ATP binding site, which is located on the inner sides of the GroE cylinder. The apical domains form the substrate and GroES binding sites. The intermediate domain connects the equatorial and apical domains of each subunit and transfers the ATP-induced conformational changes from the equatorial to the apical domain (Braig *et al.*, 1994; Xu *et al.*, 1997).

GroES is a seven-membered ring structure composed of identical 10-kDa subunits and binds in the ATP-driven chaperone cycle to one or both ends of the GroEL cylinder. GroES exhibits a dome-shaped structure with outside dimensions of 70–80 Å in diameter and a height of 30 Å and inside dimensions of 30 Å in diameter and 20 Å in height (Hunt *et al.*, 1996; Mande *et al.*, 1996). Each subunit has a β-barrel structure with two β-hairpins loops, one of which is directed outward at the top of the dome, enclosing the structure. The other, unstructured loop is located at the bottom rim of the GroES heptamer. This highly flexible loop is responsible for the interaction of GroES with GroEL and becomes ordered in the X-ray structure of the GroEL14/GroES7/ADP7 complex (Xu *et al.*, 1997).

STRUCTURAL ASPECTS OF GroE FUNCTION

The GroE chaperone machinery mediates the folding of a large number of proteins *in vivo* and *in vitro* (Viitanen *et al.*, 1992; Houry *et al.*, 1999). Following the landmark paper by Goloubinoff *et al.* (1989), the reaction cycle of GroE has been dissected *in vitro* in past years. Three different functions of the GroE system can be distinguished in this context: (i) GroE prevents the aggregation of nonnative polypeptides by forming complexes with them, thus lowering the concentration of aggregation-prone proteins in solution (Buchner *et al.*, 1991). (ii) Release of bound substrates into the central cavity of GroE complexes allows folding in a protected environment without intermolecular interactions (Weissman *et al.*, 1995, 1996; Mayhew *et al.*, 1996). (iii) GroE seems to be able to unfold kinetically trapped folding intermediates, thus giving them a new chance to fold correctly (Todd *et al.*, 1994; Sparrer *et al.*, 1997; Shtilerman *et al.*, 1999).

To perform the tasks of polypeptide binding, un-

[1] To whom correspondence should be addressed at Institut für Organische Chemie und Biochemie, Technische Universität München, Lichtenbergstrasse 4, 85747 Garching, Germany. Fax: +49 89 289 13345. E-mail: johannes.buchner@ch.tum.de.

1047-8477/01 $35.00

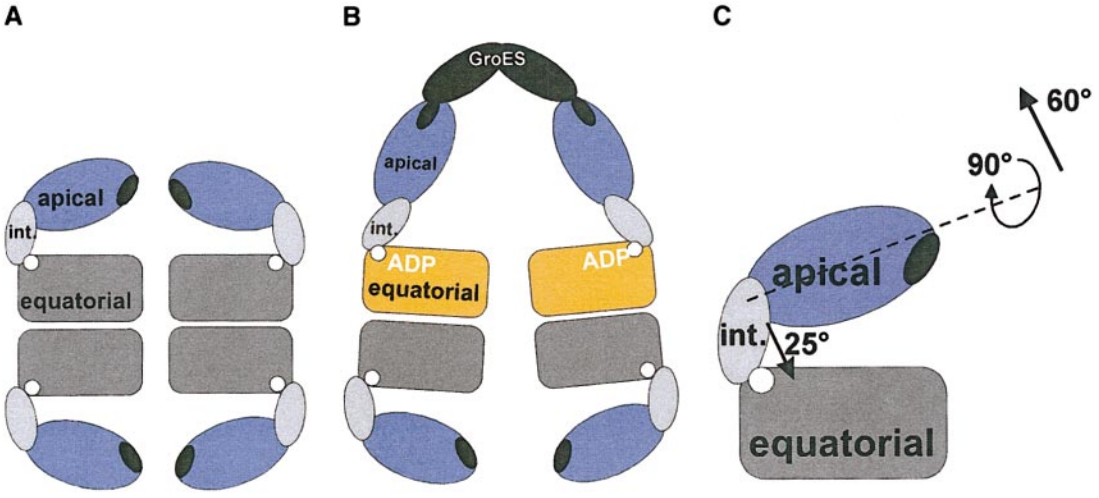

FIG. 1. Schematic representation of GroEL and GroEL in complex with GroES and ADP. (A) Representation of GroEL without bound ligands. The apical, intermediate, and equatorial domains are shown in blue, light gray, and dark gray. The substrate binding sites in the apical domains are highlighted in black. ATP binding sites in the equatorial domains are shown as white circles. (B) GroEL in complex with GroES and ADP. GroES is shown in black. The apical domain with bound ADP is highlighted in orange. (C) Schematic representation of the direction and magnitude of the domain movement within the *cis* ring (adapted from Xu *et al.*, 1997).

folding, release of bound polypeptides into the central cavity of GroE, and the ejection of sequestered proteins into solution, GroEL must adopt different conformations during the ATP-driven reaction cycle.

1. Polypeptide binding. GroEL binds a broad spectrum of unfolded or partially unfolded proteins but exhibits only low affinity for native polypeptide chains. Binding occurs mainly via hydrophobic interactions (Fenton *et al.*, 1994; Braig *et al.*, 1994). This explains the low substrate specificity and the high selectivity for unfolded substrate proteins, because in native proteins hydrophobic residues are usually buried inside the core. To some extent electrostatic interactions also contribute to substrate binding (Richarme and Kohiyama, 1994; Katsumata *et al.*, 1996; Perrett *et al.*, 1997). Polypeptide binding occurs at the inner top rim of the apical domains as shown by electron microscopy, mutagenesis, and X-ray crystallography (Braig *et al.*, 1993; Fenton *et al.*, 1994; Chatellier *et al.*, 1999; Chen and Sigler, 1999). Polypeptides bind in the groove formed by a pair of parallel α-helices of the apical domain. The structure of this region is flexible as indicated by high-temperature factors (Braig *et al.*, 1994, 1995; Boisvert *et al.*, 1996) and differences in the structures of unliganded GroEL, GroEL/peptide complexes, or GroEL in complex with GroES and ADP (Xu *et al.*, 1997; Buckle *et al.*, 1997; Chen and Sigler, 1999). This flexibility of the substrate binding site seems to be a prerequisite for the tight binding of a broad spectrum of different proteins. Importantly, the cooperative binding of a substrate polypeptide or of GroES to more than one apical domain binding site seems to increase the affinity between GroEL and its binding partner significantly (Chen and Sigler, 1999; Chatellier *et al.*, 2000).

2. Nucleotide and GroES binding. In the presence of nucleotide and GroES, GroEL shows a complex allosteric behavior (Yifrach and Horovitz, 1995), due to conformational changes in the GroEL domains. ATP binding to one GroEL ring occurs very fast (diffusion controlled) with positive cooperativity and allows subsequent GroES binding. The structure of the GroEL/GroES/ADP complex shows that the intermediate domain moves slightly inward and the apical domain moves 60° upward with a 90° torsion (see Fig. 1; Xu *et al.*, 1997). This lowers the affinity for nonnative polypeptides in this ring drastically because the substrate binding sites move from the inner rim of the apical domains toward the top of the GroEL cylinder. The conformational changes result in the doubling of the volume of the GroEL cavity (Xu *et al.*, 1997) and the release of a bound substrate protein into the cavity, and, at the same time, they enable the binding of GroES to the former substrate binding sites (Xu *et al.*, 1997). Thus, a bound substrate protein becomes sequestered in the central cavity of a GroEL14/GroES7/ATP7 complex, a so-called "*cis* complex" or "*cis* bullet" (Fig. 1).[2] This complex is a folding active species in the GroE cycle

[2] "*cis*" refers to the localization of the other ligands of a GroEL/GroES double-ring complex. If the ligands are attached to the same ring as GroES, they are *in cis;* if they are on the opposite ring to a bound GroES molecule, they are "*in trans.*"

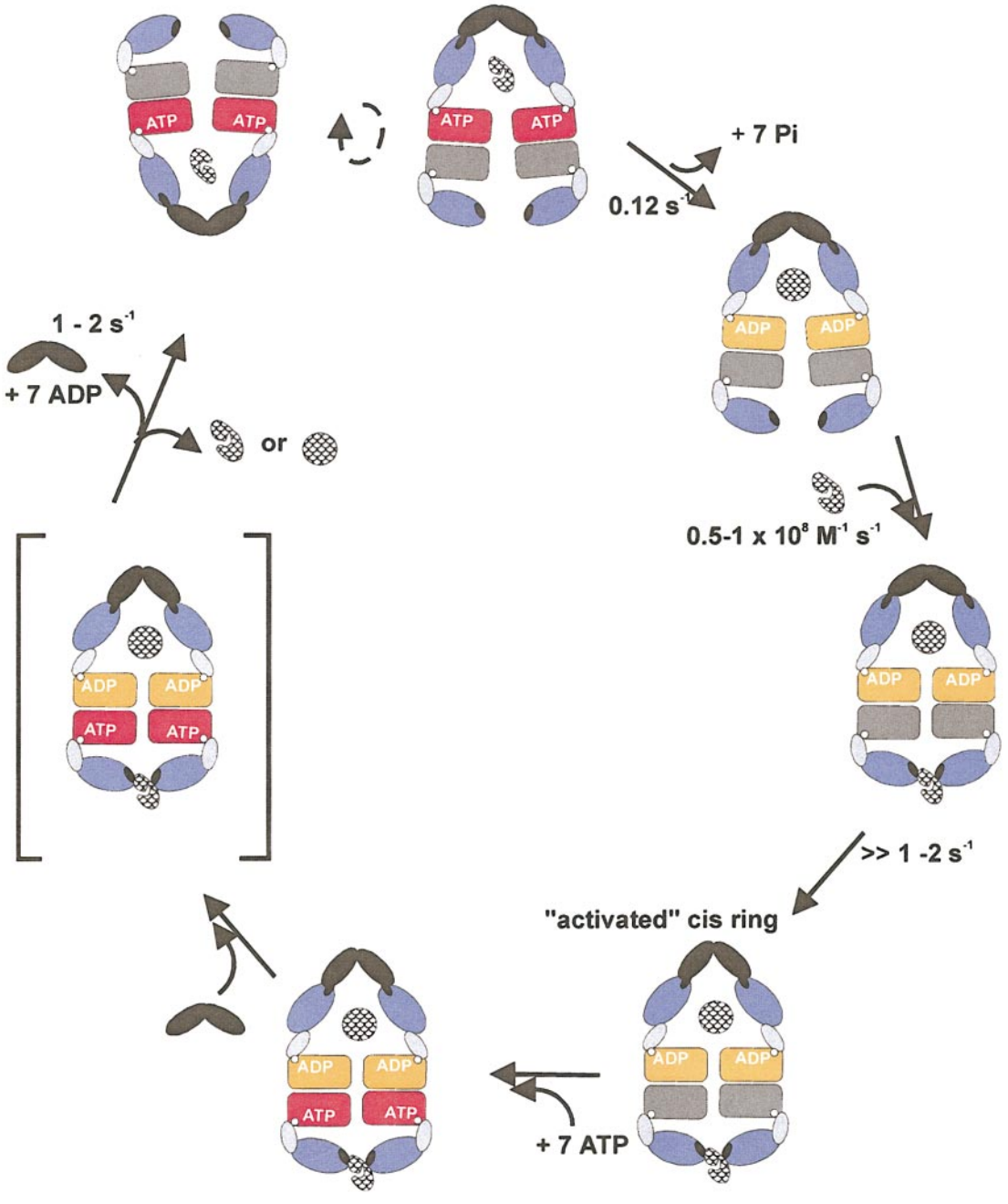

FIG. 2. Proposed model for the GroE cycle in the presence of substrate (cf. Rye *et al.,* 1999, and text). In this cycle the conversion of the ATP bullet (apical domains highlighted in red) with sequestered substrate inside to the ADP bullet (apical domains highlighted in orange) is the rate-limiting step. The switching of the folding active *cis* complexes between the rings is efficiently coupled to polypeptide binding by a structural transition of the *cis* ADP complex, resulting in an "activated" *cis* ring. Subsequently, ATP binding *in trans* triggers the release of GroES and substrate from the *cis* ring and allows binding of GroES to the *trans* ring in a concerted action (indicated by brackets). The substrate protein (irregular symbol and circle) can fold inside the *cis* chamber and can be released either in a committed or native state (circle) or in a folding state (irregular symbol) (adapted from Rye *et al.,* 1999).

(Weissman *et al.*, 1995, 1996; Mayhew *et al.*, 1996). The association of GroES to a GroEL/ATP7 ring is very fast ($>4 \times 10^7$ M^{-1} s^{-1}) (Burston *et al.*, 1995). During this rearrangement the walls of the cavity change their character from hydrophobic to hydrophilic, thus giving polypeptides the chance to fold

without intermolecular interactions (Xu *et al.*, 1997). Furthermore, negative cooperativity between the two GroEL rings prevents the binding of ATP and GroES to the opposite ring as long as ATP is bound in one ring (Yifrach and Horovitz, 1995; Burston *et al.*, 1995). The binding of a substrate protein to the *trans* ring is not affected.

3. ATP binding and hydrolysis. ATP hydrolysis is potassium dependent (Viitanen *et al.*, 1990) and occurs in an asymmetric GroEL14/GroES7/ATP7 complex at a rate of 0.12 s^{-1}, leading to the formation of a GroEL14/GroES7/ADP7 complex. ATP hydrolysis triggers these complexes to move on in the GroE reaction cycle because the negative cooperativity for ATP binding in the second ring is abolished. This leads to the formation of GroEL7/ATP7/GroEL7/GroES7/ADP7 complexes.

4. GroES and ADP release. The release of bound ADP and GroES is induced by the binding of ATP to the opposite GroEL ring (Burston *et al.*, 1995; Rye *et al.*, 1997; Kad *et al.*, 1998). Thus, ATP binding induces conformational changes, which are transferred to the other ring via the equatorial domains. These rigid body movements in the GroE complexes seem to be rate limiting in the GroE ATPase cycle leading to the subsequent release of GroES and ADP with an apparent rate of 0.042 s^{-1} (Burston *et al.*, 1995; Rye *et al.*, 1997, 1999; Kad *et al.*, 1998). Rye *et al.* (1999) presented evidence for an additional fast step in the GroES release of the *cis* bullet ($k = 2$ s^{-1}). It was suggested that the slow, rate-limiting step is a structural transition of the ADP bullet and the following fast step should correspond to the release of GroES. Furthermore, Sparrer and Buchner (1997) showed that the binding of ATP to the *trans* ring of a preformed ADP bullet occurs very fast (diffusion limited) and is promoted by GroES in the opposite GroEL ring. However, the conformational switch of the *trans* ring from a high-affinity state for substrate binding to the low-affinity state is slow, with a rate of 30 s^{-1} (Sparrer and Buchner, 1997). Thus, the binding of a substrate protein to a *trans* ring with high substrate affinity should occur well before the conformational switch to the low-affinity state.

Completion of the ATPase cycle takes about 15 s at room temperature (Todd *et al.*, 1994; Burston *et al.*, 1995). Thus, ATP hydrolysis can be regarded as a timer function leading to the discharge of polypeptides every 15 s, irrespective of their folding states. The ATPase activity of the GroE system increases linearly with temperature (Mendoza *et al.*, 1996; Grallert *et al.*, 2000). This leads to an acceleration of the timer function at physiological temperatures.

ASSOCIATION OF GroES AND GroEL

The association of GroES with GroEL follows the diffusion-controlled binding of ATP at a rate of $>4 \times 10^7$ M^{-1} s^{-1}. This reaction is concentration dependent and in the range of the association rate constants for substrate binding to GroEL (Burston *et al.*, 1995). In contrast, the association of GroES with an asymmetric GroEL14/GroES7/ADP7 complex is not identical to that of unliganded GroEL. The kinetics of GroES binding to the *trans* side of a GroEL14/GroES/ADP7 bullet is now concentration independent and occurs with an apparent rate for a unimolecular reaction of approximately 1 s^{-1}, suggesting that a unimolecular process, most likely in the ADP bullet itself, is rate limiting (Rye *et al.*, 1999). This is in good agreement with the finding that the negative cooperativity between the two GroEL rings strongly increases in the presence of GroES. Interestingly, the presence of bound substrate *in trans* does not influence the binding of ATP and GroES to that ring but accelerates the release of GroES and ADP from the opposite GroEL ring, so that ATP hydrolysis becomes the rate-limiting step in the GroE ATPase cycle (Rye *et al.*, 1999). It should be noted that based on the rate constants determined in these experiments the second GroES attaches to the *trans* ring only when the GroES bound to the *cis* ring has already departed.

IMPLICATIONS FOR THE GroE CYCLE

In previous models for the GroE reaction cycle, two ATP hydrolytic steps, one in each ring, were thought to be necessary to perform one reaction cycle (Burston *et al.*, 1995). More recent experiments have shown that for the release of GroES and ADP from GroEL14/GroES7/ADP7 complexes, the binding of ATP to the opposite ring is sufficient (see Fig. 2; Kad *et al.*, 1998; Rye *et al.*, 1999). ATP hydrolysis in a GroEL14/GroES7/ATP7 complex occurs at a rate of 0.12 s^{-1}. In a former model the slowest step in the GroE cycle was supposed to be the release of GroES from the resulting GroEL14/GroES7/ADP7 complex, which occurs after ATP binding to the opposite ring (Burston *et al.*, 1995). The experiments by Rye *et al.* (1999) suggest that a structural transition of the ADP bullet after ATP binding to the *trans* ring is the slowest step in the cycle (0.042 s^{-1}), which is followed by a fast release of GroES (2 s^{-1}). The association of GroES with the *trans*-sided ATP ring occurs with a rate constant very similar to that of the GroES release in the opposite GroEL ring (1–2 s^{-1}) (see Fig. 2).

In the presence of substrate protein in the *trans* position, the rate of *cis* complex dissociation is strongly accelerated. GroES is released from *cis* at a

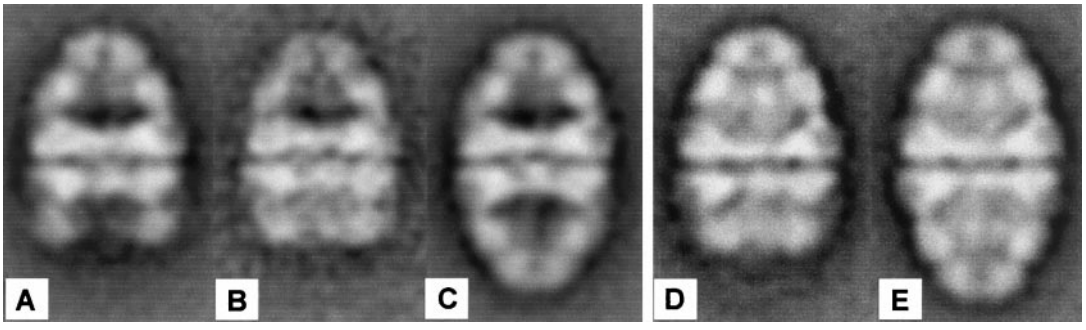

FIG. 3. Processed electron microscopic images of GroE complexes with or without substrate in the central cavity. (A) Bullet without substrate *in cis* and an open conformation *in trans.* (B) Bullet in a closed conformation *in trans.* (C) Symmetric GroEL14/(GroES7)2 "football" complex. (D) Bullet with substrate in the *cis* cavity. (E) Football with one substrate in each *cis* cavity (adapted from Beißinger *et al.,* 1999; and Grallert *et al.,* 2000).

rate of 1–2 s^{-1} (Rye *et al.,* 1999). In this case, binding of a polypeptide to the *trans* side seems to accelerate the structural changes in an ADP bullet. As a consequence, ATP hydrolysis becomes rate limiting in the cycle. As substrate binding to the *trans* ring occurs before GroES binding, the transition from one folding active *cis* complex to the next is ensured (Sparrer and Buchner, 1997; Rye *et al.,* 1999).

It has been suggested that the transition from one asymmetric GroEL14/GroES7/nucleotide7-complex to another bullet proceeds mainly via free GroEL rings unliganded with GroES (Hayer-Hartl *et al.,* 1995). The formation of a symmetrical GroEL7/GroES7/ADP/GroEL7/GroES7/ATP7 intermediate seemed to be unlikely, due to the rate constants of the partial reactions (Fig. 2) (Burston *et al.,* 1995; Rye *et al.,* 1997, 1999; Kad *et al.,* 1998).

An alternative model for the GroE cycle involves complexes consisting of GroEL with two GroES rings bound (Todd *et al.,* 1994; Azem *et al.,* 1994, 1995; Sparrer and Buchner, 1997; Sparrer *et al.,* 1997). This model is based on the analysis of GroE particles by electron microscopy and on folding experiments performed with stringent substrate proteins. These studies show clearly an increased efficiency of folding only under conditions where symmetrical GroE complexes are detected. These football complexes are transient intermediates in the reaction cycle, which are not strictly obligatory but necessary for maximum efficiency in GroE-assisted folding (see below and Fig. 5) (Azem *et al.,* 1995; Diamant *et al.,* 1995; Sparrer *et al.,* 1997; Beißinger *et al.,* 1999).

FOOTBALLS AND BULLETS

Electron microscopy and image processing showed that in the presence of ADP, asymmetric, bullet-shaped particles are formed (Langer *et al.,* 1992; Llorca *et al.,* 1994; Schmidt *et al.,* 1994). In the presence of either nonhydrolyzable ATP analogues or ATP, both asymmetric and symmetric GroE complexes were detected (see Fig. 3; Harris *et al.,* 1994; Schmidt *et al.,* 1994; Llorca *et al.,* 1994, 1996; Azem *et al.,* 1994, 1995). Subsequently, using analytical ultracentrifugation it was shown that the two GroEL rings bind GroES simultaneously (Behlke *et al.,* 1997). This is in agreement with the notion that football complexes are only apparently symmetric. The number of football complexes detected by electron microscopy and image processing amounts to up to 80% of processed GroE particles (Schmidt *et al.,* 1994; Harris *et al.,* 1994; Azem *et al.,* 1994; Llorca *et al.,* 1996). Football complexes are significantly populated under a variety of solvent condi-

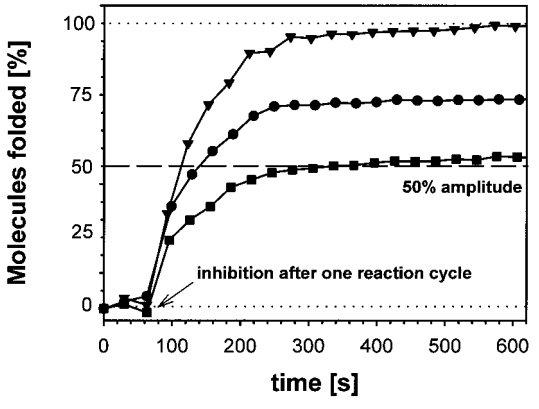

FIG. 4. Variation of the GroES to GroEL ratio in MBP folding experiments (Beißinger *et al.,* 1999). The MBP to GroEL ratio was 2:1. The kinetics show the increase of folding amplitudes at a GroEL to GroES ratio of either (■) 1:1 or (●) 1:2. The GroE-assisted folding reaction was started after 60 s by the addition of ATP and subsequently quenched after one reaction cycle by the addition of apyrase. For details of the experiment see Beißinger *et al.* (1999). (▼) shows the control of the GroE catalyzed MBP folding in the absence of the apyrase quench (adapted from Beißinger *et al.,* 1999).

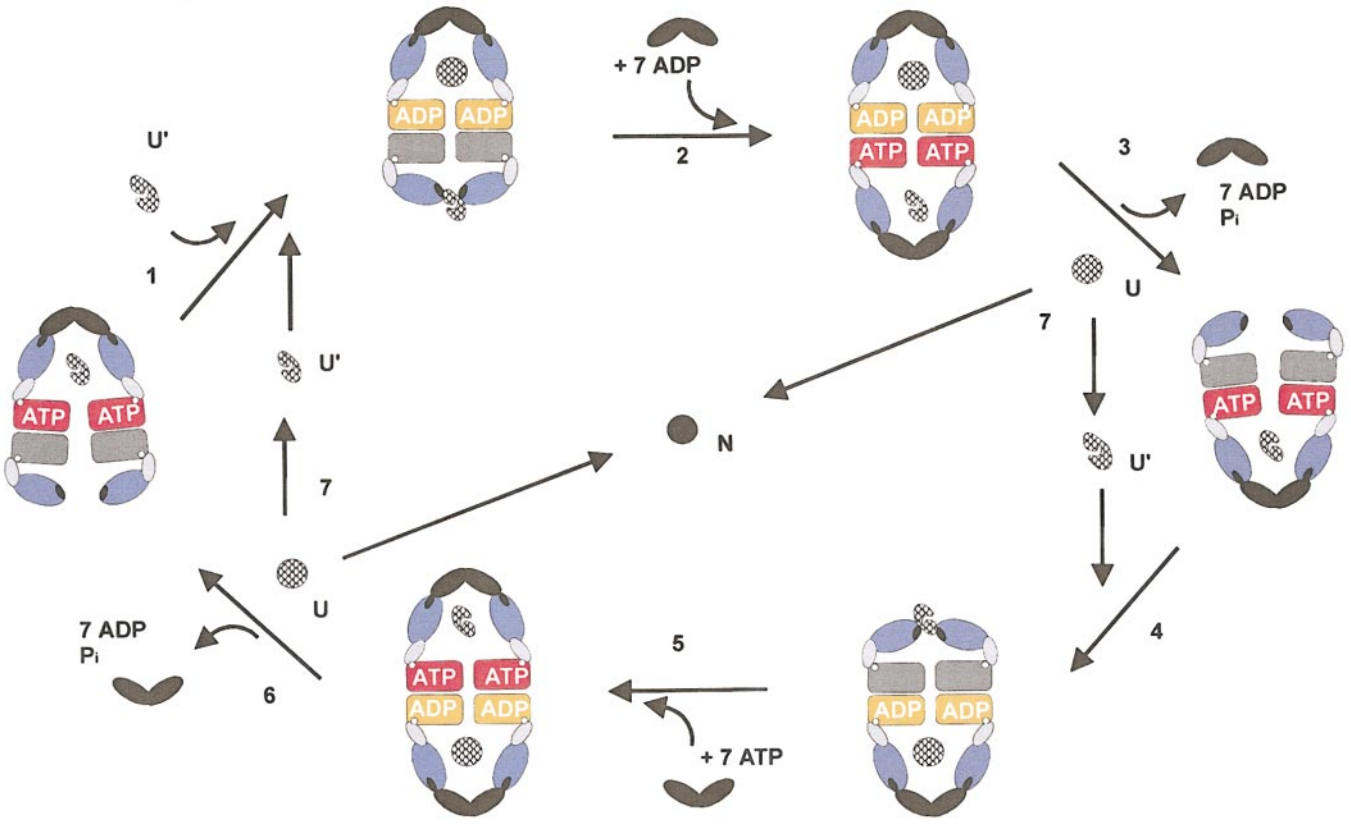

FIG. 5. Football-bullet model for the reaction cycle of GroE. A *cis*-bullet particle with GroES, ADP, and nonnative protein (irregular symbol; U′) attached to one GroEL ring binds a second folding intermediate to the high-affinity binding site of the *trans* ring (step 1). In the next step, ATP and a second GroES associate to form a football-shaped particle (step 2), encapsulating two folding intermediates. Hydrolysis of ATP in the lower GroEL ring releases GroES, ADP, and protein from the upper ring (step 3), thus completing the cycle and restoring a new high-affinity acceptor state, which is now in a rotated state. The released nonnative protein (which folded from U′ to U *in cis;* step 1) can either fold to the native state (N; step 7) or rebind to the chaperone (step 1). Reaction steps 4–6 are equivalent to steps 1–3 (adapted from Sparrer *et al.,* 1997).

tions such as different salt concentrations or temperatures (Azem *et al.,* 1994; Diamant *et al.,* 1995; Llorca *et al.,* 1996; Sparrer *et al.,* 1997; Beißinger *et al.,* 1999; Grallert *et al.,* 2000). Notably, bricks (unliganded GroEL) were present only at a few percent in these studies. This is in agreement with the finding that GroES alternates dynamically between the two ends of GroEL (Martin *et al.,* 1993). An initial caveat concerning the significance of football particles was that the conditions used in the experiments, especially pH and Mg^{2+} concentration, were not physiological (Engel *et al.,* 1995). However, in subsequent electron microscopic studies it was shown that symmetric complexes can also be detected at physiological pH values and in the presence of low Mg^{2+} concentrations. Under these conditions, 58% of the complexes were football-shaped, whereas no bricks were detected (Beißinger *et al.,* 1999). Further studies by Llorca *et al.* (1996) revealed that the number of footballs strongly depends on the K^+ concentration and on the ATP to ADP ratio. Low K^+ concentrations as well as an excess of ADP over ATP disfavor the formation of symmetric complexes. However, in the presence of sufficient ATP and K^+, symmetric complexes were formed even at a GroEL to GroES ratio of 4:1. Taken together, these studies led to the conclusion that football particles form readily under a large variety of experimental conditions, whereas bricks are not populated (Schmidt *et al.,* 1994; Azem *et al.,* 1994, 1995; Llorca *et al.,* 1996; Beißinger *et al.,* 1999). These results strongly suggest that the GroE folding cycle does not switch from one asymmetric complex to another via GroEL particles unliganded with GroES but via GroEL molecules liganded with two GroES molecules. This suggestion was further supported by the demonstration of symmetric complexes with encapsulated substrate proteins in both cavities (Llorca *et al.,* 1997; Sparrer *et al.,* 1997). It should be noted that football complexes are only

apparently symmetric as they differ in the nucleotide composition in the GroEL rings. One ring has ATP bound while the other is in the ADP state. This is in agreement with fluorescence anisotropy studies analyzing the binding stoichiometry of pyrene-labeled GroES to GroEL in the presence of mixed nucleotides. These experiments showed that only one GroES molecule binds tightly to GroEL in the presence of ADP or AMP-PNP. However, in the presence of a mixture of these nucleotides, both GroEL rings can be titrated with GroES molecules (Gorovits *et al.*, 1997). The same result was obtained in the presence of ATP. Analysis of the stability of ADP bullets and the exchange of the bound GroES with GroES in solution led to the conclusion that also in the presence of only ADP, the exchange of GroES occurs via transiently formed symmetric GroE complexes (Horowitz *et al.*, 1999).

Kinetic studies on GroE-mediated refolding of substrate proteins have shown a correlation between the efficiency of refolding and the occurrence of symmetric GroEL14/GroES14 complexes (Azem *et al.*, 1995; Sparrer *et al.*, 1997; Ben-Zvi *et al.*, 1998; Beißinger *et al.*, 1999). Direct evidence for the involvement of football complexes in GroE-dependent folding was obtained using a folding mutant of maltose binding protein (MBP) as a substrate for GroE. The folding process of this mutant is decelerated compared to the wild-type protein (Chun *et al.*, 1993). It turned out that the GroE system accelerated the folding process up to 30-fold (Sparrer *et al.*, 1997). Importantly, maximum catalysis was obtained only at GroES to GroEL ratios at which footballs were maximally populated (Sparrer *et al.*, 1997). This correlation between the increase in folding efficiency and the GroES concentration can be explained only by the faster and more efficient conversion of asymmetric *cis* complexes via symmetric complexes. Further studies on the folding of MBP revealed that within one GroE cycle the folding of up to 75% of the MBP molecules was accelerated in the presence of football particles (Beißinger *et al.*, 1999). In the presence of bullets only, it is impossible to fold more than 50% of the bound MBP in one round (see Fig. 4; cf. Beißinger *et al.*, 1999). This result highlights the importance of symmetric particles in the GroE cycle (Fig. 5). The binding of two substrate proteins simultaneously ensures an efficient flip–flop mechanism of binding, release in the cavity, and ejection into solution, with the two rings of GroEL simultaneously active in protein folding (Sparrer *et al.*, 1997). Folding studies using barnase as a substrate protein also led to the conclusion that in the presence of GroES:GroEL ratios higher than 1, symmetric complexes that increase the efficiency of folding were formed transiently (Corrales and Fersht, 1996). Further analysis of the folding of barnase and mMDH showed that the maximum rates of folding for these proteins could be achieved at molar ratios of GroES:GroEL oligomers of 2:1 and greater (Ben-Zvi *et al.*, 1998). Thus the involvement of symmetric complexes seems to represents a general mechanism for efficient folding of stringent substrate proteins.

FOOTBALLS *IN VIVO*

Interestingly, most of the studies concerning the involvement of symmetric GroE complexes in the folding of substrate proteins were done under nonpermissive folding conditions (Azem *et al.*, 1995; Sparrer *et al.*, 1997; Beißinger *et al.*, 1999). Since the negative cooperativity between the two GroEL rings decreases with increasing temperatures (Llorca *et al.*, 1998; Terada and Kuwajima, 1999) the release of GroES from GroEL is decelerated (Llorca *et al.*, 1998). This implies that the association of a second GroES with an asymmetric GroEL/GroES complex is more likely at higher than at lower temperatures. The physiological growth temperature of *Escherichia coli* is 37°C. Therefore the strong negative cooperativity between the two GroEL rings that disfavors the formation of symmetric GroE complexes should be decreased *in vivo*. Furthermore, the concentration of GroEL in the cell is high, around 2.5 μM (cf. Lorimer, 1996). Given the rate constants for the formation of GroE complexes (Behlke *et al.*, 1997; Gorovits *et al.*, 1997), these concentrations are clearly sufficient to allow association of two GroES molecules to GroEL. Interestingly in *E. coli*, the *groEL* and *groES* genes are arranged in an operon from which they are transcribed in a coordinated manner, resulting in a 1:1 ratio of expressed subunits or a GroEL14 to GroES7 ratio of 1:2 (cf. Lorimer, 1996). Furthermore, the nucleotide and salt concentrations in *E. coli* correspond well to those employed in the *in vitro* studies. Taken together, structural and kinetic studies over the past years have provided compelling evidence that football particles are transient intermediates of the GroE chaperone cycle mediating the switch from one bullet to another.

We thank Stefan Walter and Kerstin Rutkat for stimulating discussions. Work in the authors' laboratory was supported by grants from the German–Israeli Science Foundation, DFG, BMBF, and the Fonds der chemischen Industrie.

REFERENCES

Azem, A., Kessel, M., and Goloubinoff, P. (1994) Characterisation of a functional GroEL14(GropES7)2 chaperonin hetero-oligomer. *Science* **265,** 653–656.

Azem, A., Diamant, S., Kessel, M., Weiss, C., and Goloubinoff, P.

(1995) The protein-folding activity of chaperonins correlates with the symmetric GroEL14(GroES7)2 heterooligomer. *Proc. Natl. Acad. Sci. USA* **92,** 12021–12025.

Behlke, J., Ristau, O., and Schönfeld, H.-J. (1997) Nucleotide-dependent complex formation between the *Escherichia coli* chaperonins GroEL and GroES studied under equilibrium conditions. *Biochemistry* **36,** 5149–5156.

Beißinger, M., Rutkat, K., and Buchner, J. (1999) Catalysis, commitment and encapsulation during GroE-mediated folding. *J. Mol. Biol.* **289,** 1075–1092.

Ben-Zvi, A. P., Chatellier, J., Fersht, A. R., and Goloubinoff, P. (1998) Minimal and optimal mechanisms for GroE-mediated protein folding. *Proc. Natl. Acad. Sci. USA* **95,** 15275–15280.

Boisvert, D. C., Wang, J., Otwinowski, Z., Horwich, A. L., and Sigler, P. B. (1996) The 2.4 Å crystal structure of the bacterial chaperonin GroEL complexed with ATPγS. *Nat. Struct. Biol.* **3,** 170–177.

Braig, K., Simon, M., Furuya, F., Hainfeld, J. F., and Horwich, A. L. (1993) A polypeptide bound by the chaperonin GroEL is localised within a central cavity. *Proc. Natl. Acad. Sci. USA* **90,** 3978–3982.

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 resolution. *Nature* **371,** 578–586.

Braig, K., Adams, P. T., and Brünger, A. T. (1995) Conformational variability in the refined structure of the chaperonin GroEL at 2.8 Å resolution. *Nat. Struct. Biol.* **2,** 1083–1094.

Buchner, J., Schmidt, M., Fuchs, M., Jaenicke, R., Rudolph, R., Schmid, F. X., and Kiefhaber, T. (1991) GroE facilitates the refolding of citrate synthase by suppressing aggregation. *Biochemistry* **30,** 1586–1591.

Buckle, A. M., Zahn, R., and Fersht, A. R. (1997) A structural model for GroEL-polypeptide recognition. *Proc. Natl. Acad. Sci. USA* **94,** 3571–3575.

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.

Chatellier, J., Hill, F., and Fersht, A. R. (2000) From minichaperone to GroEL 2: Importance of avidity of the multisite ring structure. *J. Mol. Biol.* **15,** 883–896.

Chatellier, J., Buckle, A. M., and Fersht, A. R. (1999) GroEL recognises sequential and nonsequential linear structural motifs compatible with extended beta-strands and alpha-helices. *J. Mol. Biol.* **292,** 163–172.

Chen, L., and Sigler, P. B. (1999) The crystal structure of a GroEL/peptide complex: Plasticity as a basis for substrate diversity. *Cell* **99,** 757–768.

Chen, S., Roseman, A. M., Hunter, A. S., Wood, S. P., Burston, S. G., Ranson, N. A., Clarke, A. R., and Saibil, H. R. (1994) Location of a folding protein and shape changes in GroEL–GroES complexes imaged by cryo-electron microscopy. *Nature* **371,** 261–264.

Chun, S.-Y., Strobel, S., Bassford, P., Jr., and Randall, L. L. (1993) Folding of maltose-binding protein. *J. Biol. Chem.* **268,** 20855–20862.

Corrales, F. J., and Fersht, A. R. (1996) Kinetic significance of GroEL14(GroES7)2 complexes in molecular chaperone activity. *Folding Design* **1,** 265–273.

Diamant, S., Azem, A., Weiss, C., and Goloubinoff, P. (1995) Increased efficiency of GroE-assisted protein folding by manganese ions. *J. Biol. Chem.* **24,** 28387–28391.

Engel, A., Hayer-Hartl, M. K., Goldie, K. N., Pfeifer, G., Hegerl, R., Müller, S., da Silva, A. C. R., Baumeister, W., and Hartl, F. U. (1995) Functional significance of symmetrical versus asymmetrical GroEL–GroES chaperonin complexes. *Science* **269,** 832–836.

Fenton, W. A., Kashi, Y., Furtak, K., and Horwich, A. L. (1994) Residues in chaperonin GroEL required for polypeptide binding and release. *Nature* **371,** 614–619.

Goloubinoff, P., Gatenby, A. A., and Lorimer, G. H. (1989) GroE heat-shock proteins promote assembly of foreign prokaryotic ribulose bisphosphate carboxylase oligomers in *Escherichia coli*. *Nature* **337,** 44–47.

Gorovits, B. M., Ybarra, J., Seale, J. W., and Horowitz, P. M. (1997) Conditions for nucleotide-dependent GroES–GroEL interactions. *J. Biol. Chem.* **272,** 26999–27004.

Grallert, H., Rutkat, K., and Buchner, J. (2000) Limits of protein folding inside GroE complexes. *J. Biol. Chem.* **275,** 20424–20430.

Harris, J. R., Plückthun, A., and Zahn, R. (1994) Transmission electron microscopy of GroEL, GroES and the symmetrical GroEL/GroES complex. *J. Struct. Biol.* **112,** 216–230.

Hayer-Hartl, M. K., Martin, J., and Hartl, F. U. (1995) Asymmetrical interaction of GroEL and GroES in the ATPase cycle of assisted protein folding. *Science* **269,** 836–841.

Horowitz, P. M., Lorimer, G. H., and Ybarra, J. (1999) GroES in the asymmetric GroEL14–GroES7 complex exchanges via an associative mechanism. *Proc. Natl. Acad. Sci. USA* **96,** 2682–2686.

Houry, W. A., Frishman, D., Eckerskorn, C., Lottspeich, F., and Hartl, F. U. (1999) Identification of *in vivo* substrates of the chaperonin GroEL. *Nature* **402,** 147–154.

Hunt, J. F., Weaver, A. J., Samuel, J. L., Gierasch, L., and Deisenhofer, J. (1996) The crystal structure of the GroES-cochaperonin at 2.8 Å resolution. *Nature* **379,** 37–45.

Ishii, N., Taguchi, H., Sasabe, H., and Yoshida, M. (1994) Folding intermediate binds to the bottom of bullet-shaped holo-chaperonin and is readily accessible to antibody. *J. Mol. Biol.* **236,** 691–696.

Kad, N. M., Ranson, N. A., Cliff, M. J., and Clarke, A. R. (1998) Asymmetry, commitment and inhibition in the GroE ATPase cycle impose alternating functions on the two GroEL rings. *J. Mol. Biol.* **278,** 267–278.

Katsumata, K., Okazaki, A., and Kuwajima, K. (1996) Effect of GroEL on the refolding of α-lactalbumin. *J. Mol. Biol.* **258,** 827–838.

Langer, T., Pfeifer, G., Martin, J., Baumeister, W., and Hartl, F. U. (1992) Chaperonin-mediated protein folding: GroES binds to one end of the GroEL cylinder, which accommodates the protein substrate within its central cavity. *EMBO J.* **11,** 4757–4765.

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.

Llorca, O., Carrascosa, J. L., and Valpuestra, J. M. (1996) Biochemical characterisation of symmetric GroEL–GroES complexes. *J. Biol. Chem.* **271,** 68–76.

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.

Llorca, O., Galan, A., Carrascosa, J. L., Muga, A., and Valpuesta, J. M. (1998) GroEL under heat shock: Switching from a folding to a storage function. *J. Biol. Chem.* **273,** 32587–32594.

Lorimer, G. H. (1996) A quantitative assessment of the role of the

chaperonin proteins in protein folding *in vivo*. *FASEB J.* **10,** 5–9.

Mande, S. C., Mehra, V., Bloom, B. R., and Hol, W. G. J. (1996) Structure of the heat shock protein chaperonin-10 of *Mycobacterium leprae*. *Science* **271,** 203–207.

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.

Mayhew, M., da Silva, A. C. R., Martin, J., Erdjument-Bromage, H., Tempst, P., and Hartl, F. U. (1996) Protein folding in the central cavity of the GroEL–GroES chaperonin complex. *Nature* **379,** 420–426.

Mendoza, J. A., Warren, T., and Dulin, P. (1996) The ATPase activity of chaperonin GroEL is highly stimulated at elevated temperatures. *Biochem. Biophys. Res. Commun.* **229,** 271–274.

Perrett, S., Zahn, R., Sternberg, G., and Fersht, A. R. (1997) Importance of electrostatic interactions in the rapid binding of polypeptides to GroEL. *J. Mol. Biol.* **269,** 892–901.

Richarme, G., and Kohiyama, M. (1994) Amino acid specifity of the *Escherichia coli* chaperone GroEL (heat shock protein 60). *J. Biol. Chem.* **269,** 7095–7098.

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 cis and trans ATP within the double ring of the chaperonin GroEL. *Nature* **388,** 792–798.

Rye, H. S., Roseman, A. L., 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.

Schmidt, M., Rutkat, K., Rachel, R., Pfeifer, G., Jaenicke, R., Viitanen, P., Lorimer, G. H., and Buchner, J. (1994) Symmetric complexes of GroE chaperonins as a part of the functional cycle. *Science* **265,** 656–659.

Shtilerman, M., Lorimer, G. H., and Englander, W. S. (1999) Chaperonin function: Folding by forced unfolding. *Science* **284,** 822–825.

Sparrer, H., and Buchner, J. (1997) How GroES regulates binding of nonnative protein to GroEL. *J. Biol. Chem.* **272,** 14080–14086.

Sparrer, H., Rutkat, K., and Buchner, J. (1997) Catalysis of protein folding by symmetric chaperone complexes. *Proc. Natl. Acad. Sci. USA* **94,** 1096–1100.

Terada, T. P., and Kuwajima, K. (1999) Thermodynamics of nucleotide binding to the chaperonin GroEL studied by isothermal titration calorimetry: Evidence for noncooperative nucleotide binding. *Biochim. Biophys. Acta* **1431,** 269–281.

Todd, M. J., Viitanen, P. V., and Lorimer, G. H. (1994) Dynamics of the chaperonin ATPase cycle: Implications for facilitated protein folding. *Science* **265,** 659–666.

Viitanen, P. V., Lubben, T. H., Reed, J., Goloubinoff, P., Okeefe, D. P., and Lorimer, G. H. (1990) Chaperonin facilitated refolding of ribulose bis-phosphate carboxylase and ATP hydrolysis by chaperonin-60 (GroEL) are K^+ dependent. *Biochemistry* **29,** 5665–5671.

Viitanen, P. V., Gatenby, A. A., and Lorimer, G. H. (1992) Purified chaperonin-60 (GroEL) interacts with the nonnative states of a multitude of *Escherichia coli* proteins. *Protein Sci.* **1,** 363–369.

Weissman, J. S., Hohl, C. M., Kovalenke, O., Kashi, Y., Chen, S., Braig, K., Saibil, H. R., Fenton, W. A., and Horwich, A. L. (1995) Mechanism of GroEL-action—Productive release of polypeptide from a sequestered position under GroES. *Cell* **83,** 577–587.

Weissman, J. S., Rye, H. S., Fenton, W. A., Beechem, J. M., and Horwich, A. L. (1996) Characterisation of the active intermediate of a GroEL–GroES-mediated protein folding reaction. *Cell* **84,** 481–490.

Xu, Z., Horwich, A. L., and Sigler, P. B. (1997) The crystal structure of the asymmetric GroEL–GroES–ADP97 chaperonin complex. *Nature* **388,** 741–750.

Yifrach, O., and Horovitz, A. (1995) Nested cooperativity in the ATPase activity in the oligomeric chaperonin GroEL. *Biochemistry* **34,** 5303–5308.