CMLS, Cell. Mol. Life Sci. 59 (2002) 1589–1597
1420-682X/02/101589-09

CMLS Cellular and Molecular Life Sciences

Structure and function of the GroE chaperone

S. Walter

Institut für Organische Chemie and Biochemie, Technische Universität München, Lichtenbergstr. 4, 85747 Garching (Germany), Fax +49 89 289 13345, e-mail: stefan.walter@ch.tum.de

Abstract. The *Escherichia coli* proteins GroEL and GroES were the first chaperones to be studied in detail and have thus become a role model for assisted protein folding in general. A wealth of both structural and functional data on the GroE system has been accumulated over the past years, enabling us now to understand the basic principles of how this fascinating protein-folding machine accomplishes its task. According to the current model, GroE processes a nonnative polypeptide in a cycle consisting of three steps. First, the polypeptide substrate is captured by GroEL. Upon binding of the cochaperone GroES and ATP, the substrate is then discharged into a unique microenvironment inside of the chaperone, which promotes productive folding. After hydrolysis of ATP, the polypeptide is released into solution. Moreover, GroE may actively increase the folding efficiency, e.g. by unfolding of misfolded protein molecules. The mechanisms underlying these features, however, are yet not well characterized.

Key words. Molecular chaperones; protein folding; GroEL; aggregation; protein structure.

Introduction

Historically, the GroE proteins of *Escherichia coli* were the first chaperone proteins to be studied on a molecular level [1, 2]. In the early 1970s, temperature-sensitive, mutant *E. coli* strains were isolated that were unable to support the growth of bacteriophage λ [1]. Further analyses revealed that apparently two host proteins, GroEL (57 kDa) and GroES (10 kDa), were required for the correct assembly of the phage capsids. Both proteins were found to be essential for the growth of *E. coli* [3]. At that time, the cellular function of GroEL was unknown. The turning point came in the late 1980s, when George Lorimer and co-workers began to investigate whether the GroE proteins could assist in the biogenesis of Rubisco expressed in *E. coli* [4]. They observed that in wild-type cells the formation of active Rubisco was severely compromised. Upon overexpression of both GroEL and GroES, however, active Rubisco was produced. It was suggested that GroE's primary function was to prevent the aggregation of Rubisco during its folding [5], as could be demonstrated later [6, 7].

In the past decade, a wealth of both biochemical and structural data on the GroE chaperone has been accumulated, making it the most thoroughly investigated chaperone system so far [8–12]. According to the model emerging from this data, GroE-assisted protein folding is a three-step process. An aggregation-prone folding intermediate is first captured by GroEL and thereby becomes protected from aggregation. Upon binding of ATP and GroES to the GroEL/polypeptide complex, the polypeptide is ejected into a closed compartment formed by the GroE chaperone, where folding is initiated. After hydrolysis of ATP, both GroES and the polypeptide are released. While this basic mechanism of GroE action is now widely accepted, there are still a number of details that remain controversial and require further experimental investigation.

Architecture of the GroEL protein

The mechanism of GroE-mediated protein folding is intimately related with the oligomeric structure of the chaperone [13]. The GroEL molecule is a complex assembly comprising 14 identical 57-kDa subunits. The transitions between the different functional states of the chaperone are triggered by a set of domain movements which in turn are controlled by the binding of ATP and the cochaperone GroES.

The first images of the GroE chaperone were obtained by electron microscopy [14, 15]. They showed cylindrical particles containing a central channel, which could be occupied by a polypeptide substrate [16–18]. A more detailed picture became available with the X-ray structure of GroEL [19]. It confirmed that the GroEL molecule resembles a barrel with dimensions of 137 Å (diameter) and 146 Å (height). Its 14 subunits are arranged in two rings stacked back to back (fig. 1A). The two rings enclose two separate cavities (45 Å wide) that serve as folding compartments for polypeptide substrates (fig. 1B).

Each GroEL subunit can be dissected into three distinct domains (fig. 1A, C) [19]. The equatorial domains (residues 6–133 plus 409–523) constitute the central part of the cylinder and consist mainly of α helices. They serve as the foundations of the GroEL oligomer, since they mediate all interring contacts, and most of the intraring contacts. They also contain the binding pockets for ATP (fig. 1C), which are facing toward the inside of the central cavity.

In contrast to the equatorial domain, the apical domain (residues 191–376) is considerably less ordered. It is located at the opening of the GroEL cylinder (fig. 1) and contains the binding site for both GroES and the polypeptide substrate. Polypeptide binding occurs in a hydrophobic groove, which is formed by two helices facing the central channel (fig. 1C) [20, 21]. This is in agreement with results of an earlier analysis employing site-directed mutagenesis [22]. The bound substrate is stabilized by mainly hydrophobic interactions, but hydrogen bonds between its peptide backbone and the surrounding polar surface of the apical domain may contribute as well. Importantly, this hydrophobic groove is also responsible for the binding of GroES [23].

The intermediate domain (residues 134–190 plus 377–408) serves as a molecular hinge connecting the apical domain with the equatorial domain. Accordingly, its main function is the transmission of allosteric signals between both domains, thus establishing a tight coupling between nucleotide binding and GroES/polypeptide binding.

The interaction between GroES and GroEL

The cochaperone, GroES (fig. 2A), is a dome-shaped heptamer with diameter of 75 Å and a height of 30 Å [24]. It consists almost exclusively of β sheets. Residues 16–33 form the so-called mobile loops, flexible extensions that dangle from the GroES molecule like the tentacles of a jellyfish [25, 26]. Binding of GroES occurs at the apical domains of the GroEL tetradecamer (fig. 2B) and requires that the nucleotide binding sites of the respective GroEL ring are occupied with either ATP or ADP [15, 27, 28]. Upon association, the mobile loops of GroES bind to the hydrophobic peptide binding groove of GroEL and become immobilized [23, 25]. Because of the common seven-fold symmetry of both proteins, binding is thought to be highly cooperative.

Upon binding of its cochaperone, the GroEL molecule undergoes major structural rearrangements that are cen-

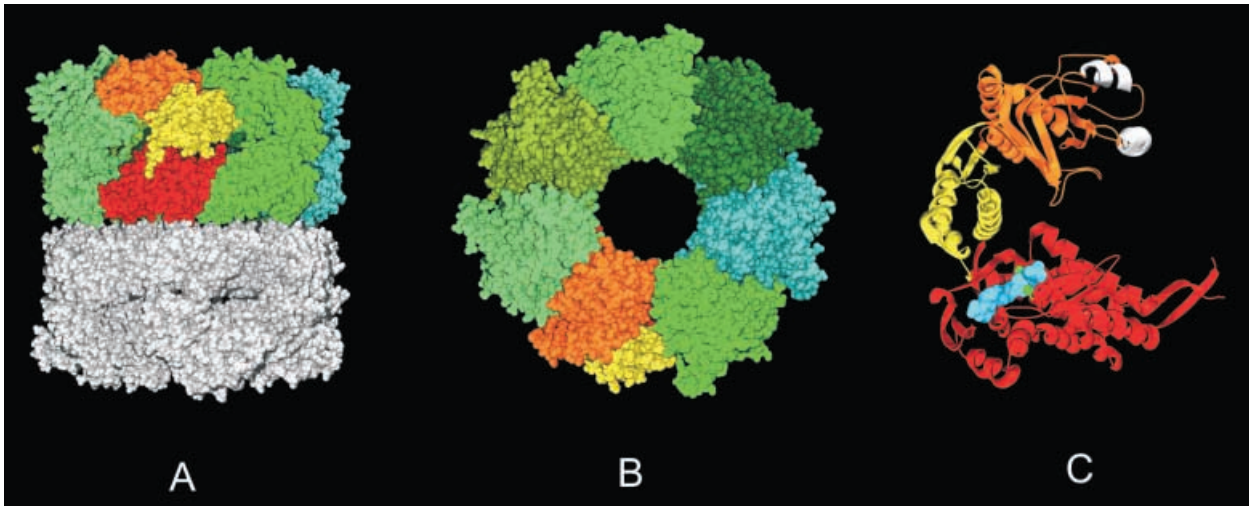

Figure 1. Structure of the GroEL chaperone from *E. coli* [19, 85]. (*A*) Side view of the GroEL tetradecamer. The particle is 137 Å wide and 146 Å high. Subunits comprising the top ring are shown in color, subunits of the bottom ring are shown in gray. Each subunit can be dissected into three domains: apical (orange), intermediate (yellow) and equatorial (red). (*B*) Top view of the GroEL tetradecamer. The diameter of the central cavity is 45 Å. The seven subunits of the ring are shown in shades of green. For one subunit, the apical and the intermediate domains are highlighted in orange and yellow, respectively. (*C*) Ribbon representation of a GroEL subunit. The equatorial domain (red) consists almost exclusively of α helices and contains the nucleotide binding site, which is occupied by ATPγS (blue). The intermediate domain (yellow) serves as a molecular hinge that connects the equatorial domain with the apical domain (orange). Binding of GroES and polypeptides occurs in a hydrophobic groove formed by the two helices (white) facing the central cavity.

tral to its functional cycle (fig. 2B, C) [23, 29]. First, the apical domains of the *cis* ring, i.e. the ring to which GroES binds, swing upward by ~60° and rotate outward by ~90°. As a result, the diameter of the central cavity almost doubles, and its volume increases from 85,000 Å^3 to 175,000 Å^3. Second, the hydrophobic residues, which form the peptide binding site of GroEL, are moved away from the cavity surface and become buried within the wall (fig. 2C). Thus, the surface of the *cis* cavity becomes largely hydrophilic. Third, GroES now blocks the exit of the cavity. As a result, the *cis* cavity is converted from an acceptor site for hydrophobic polypeptides into a closed microenvironment for protein folding.

Depending on the experimental conditions, two types of complexes between GroES and GroEL have been detected by electron microscopy. In the presence of ADP or micromolar concentrations of ATP, GroES binds to only one end of the GroEL cylinder forming asymmetric 'bullets' (see fig. 2B) [15, 16]. At ATP concentrations in the millimolar range, symmetrical 'footballs' have been observed in which both ends of the GroEL particle are capped with GroES [30–32]. It is assumed that the 'ADP bullet', i.e. the $GroES_7 \cdot ADP_7 \cdot GroEL_7/GroEL_7$ complex, represents the 'acceptor state' of GroE [33], which captures an unfolded polypeptide. The footballs presumably reflect a transient species that is formed during the functional cycle (section 6).

Polypeptide binding by GroEL

All molecular chaperones interact with unfolded or partially folded polypeptides. In the case of GroEL, a study using denatured proteins from cell extracts showed that ~40% of the *E. coli* proteins can bind to GroEL [34]. However, it is unlikely that GroEL participates in the folding of all these proteins, because its cellular concentration of ~ 1 μM is simply too small for that purpose [35]. A number of *E. coli* proteins that interact with GroEL in vivo have been identified [36], but it is not clear yet how many of them are stringently dependent on GroE in their folding.

Polypeptide binding to GroEL is primarily based on the hydrophobic effect, as was shown by a thermodynamic analysis of the binding reaction [37], although electrostatic interactions may play a role as well [38, 39]. Unlike correctly folded proteins, unfolded or partially folded polypeptides usually expose hydrophobic surfaces that can associate nonspecifically, forming higher-order aggregates [40, 41]. By binding to the hydrophobic groove in the apical do-

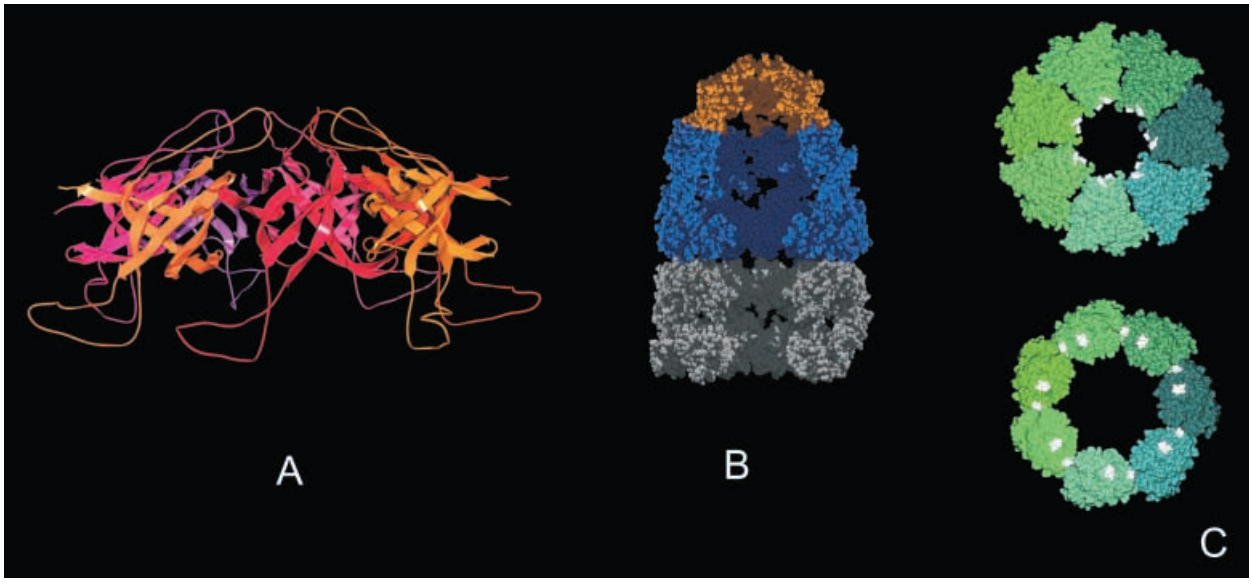

Figure 2. Structure of the GroE chaperone from *E. coli* [23, 24]. (*A*) Side view of the GroES heptamer. The individual subunits (in shades of red) consist mainly of β sheets and form a dome with a diameter of 75 Å. The flexible extensions on the bottom are the so-called mobile loops that mediate binding to GroEL. (*B*) Cross-section of a GroE 'bullet'. Each GroEL ring encloses a cavity that serves as a folding compartment for a polypeptide substrate. Some residues of the equatorial domains have not been resolved in the crystal structure, giving the wrong impression that the two cavities are contiguous. Binding of GroES (orange) to the top GroEL ring (blue) blocks the access to the upper cavity and concomitantly induces an en bloc movement of the apical domains. (*C*) Changes in the GroEL structure upon binding of GroES. In this top view, the seven subunits comprising one ring of GroEL are shown in shades of green and blue. The equatorial domains have been omitted for sake of clarity. The hydrophobic residues in the apical domains important for binding of polypeptide and GroES are shown in white. In the absence of GroES (top panel), these residues coat the inside of the central cavity and account for the high affinity for unfolded polypeptides of this state. Upon binding of GroES (lower panel) the apical domains rotate outwards by ~ 90°. The hydrophobic patches become buried in the subunit interfaces, rendering the inner surface of the cavity mainly hydrophilic and causing the release of a bound polypeptide. Concomitantly, the diameter of the cavity increases from 45 to 80 Å.

mains of GroEL (fig. 1C) these regions become shielded, and the molecule is protected from aggregation. Since most native proteins do not expose hydrophobic surfaces, GroEL will not recognize them as substrates.

The structure of various substrate proteins has been characterized while bound to GroEL. It appears that GroEL is capable of interacting with different conformations ranging from largely unfolded polypeptides to highly structured, stable folding intermediates [42–45]. It has long been a matter of debate whether GroEL recognizes certain structure motifs in its polypeptide substrates [36, 46], but most data suggest that this is not the case [47, 48]. Rather, it seems to be important whether structure formation in the polypeptide and binding to GroEL are synergistic or antagonistic processes. This is illustrated by a study using short peptides with identical amino acid composition but different sequence [49]. Only when the spacing of the hydrophobic residues was such that an amphipathic α helix could form, binding to GroEL was found to be tight. In this case, the formation of structure (= helix) and the formation of a high-affinity binding interface (= hydrophobic surface) were synergistic. Similar results were obtained for peptides mimicking β strands. It is likely that both the partially folded polypeptide and the binding site on GroEL undergo structural rearrangements upon association in order to optimize the binding interface [21, 50].

Allosteric interactions within the GroE chaperone

Though each GroEL ring consists of seven subunits, it represents a single operational unit [10, 51]. This behavior is the consequence of a framework of allosteric interactions that coordinates the binding properties of the individual subunits. There are two levels of cooperativity within the GroEL molecule. First, subunits of the same ring are subject to positive cooperativity. As an example, binding of ATP to one GroEL subunit promotes the binding of ATP to the other six subunits of the same ring [28, 52, 53]. Second, there is a negative cooperativity between the rings, i.e. binding of ATP to one ring reduces the affinity for ATP of the second ring [54, 55]. These homotropic effects can be described by a model of nested cooperativity (fig. 3) [56].

Each GroEL subunit can adopt one of two states: the (relaxed) R state, and the (tense) T state, which differ in their affinity for nucleotide and protein ligands [57]. The R state is characterized by a high affinity for ATP and a low affinity for polypeptides, whereas the T state has a low affinity for ATP and a high affinity for polypeptides. Owing to the positive intraring cooperativity, each ring is either in the R form or in the T form. Thus, the GroEL tetradecamer can adopt the configurations TT, TR, and RR (fig. 3). In the absence of nucleotides, GroEL is preferentially in the TT state. Low concentrations of ATP shift the equilibrium to the RT state, in which the R ring is completely occupied with nucleotide, whereas the T ring is empty. Because of the negative interring cooperativity, the transition to the RR state only occurs at higher ATP concentrations (>100 μM). The cochaperone GroES, on the other hand, seems to reduce the negative interring cooperativity, since its binding to the RT state promotes the transition to the RR state [57]. This is consistent with the finding that in the 'ATP bullet' complex ($GroES_7 \cdot ATP_7 \cdot GroEL_7/GroEL_7$) the trans ring shows a decreased affinity for polypeptides [58].

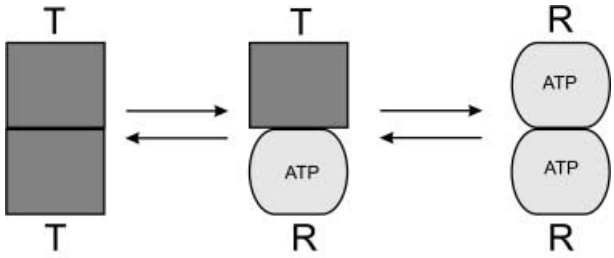

Figure 3. Model of nested cooperativity in GroEL [57]. Each rectangle (square or round) represents a single ring. Owing to the positive intraring cooperativity, all seven subunits within one ring adopt the same state. In the absence of ligands, GroEL is predominantly in the TT state (left). In the presence of low concentrations of ATP, the equilibrium is shifted towards the TR state (middle), because ATP preferentially binds to the R conformation. At higher concentrations of ATP, transition to the RR state occurs.

The functional cycle of the GroE chaperone

GroE-mediated folding requires the polypeptide substrate to participate in a cycle which can be dissected into three steps: capture, sequestration/folding and release [59]. Depending on the nature of the polypeptide, multiple rounds may be necessary for successful folding [60, 61]. The cycling ends when the polypeptide molecule has reached a conformation that is no longer recognized by GroEL. For some monomeric proteins like rhodanese, this exit point may be the native state [33, 62]. In general, however, it will be a committed state in which the protein has not yet reached its native conformation, but no longer requires the assistance of GroE [45, 63]. The reactions that lead from there to the native state may include further folding processes as well as oligomerization. Other molecules may adopt a conformation from which the native state is kinetically inaccessible. These dead-end products probably become degraded by cellular proteases [9].

The sequence of events during GroE cycling is best explained on the level of a single ring. The cycle starts when a polypeptide is captured by GroE (fig. 4, step 1). As mentioned above, a potential substrate is recognized by virtue of its exposed hydrophobic surfaces. The acceptor

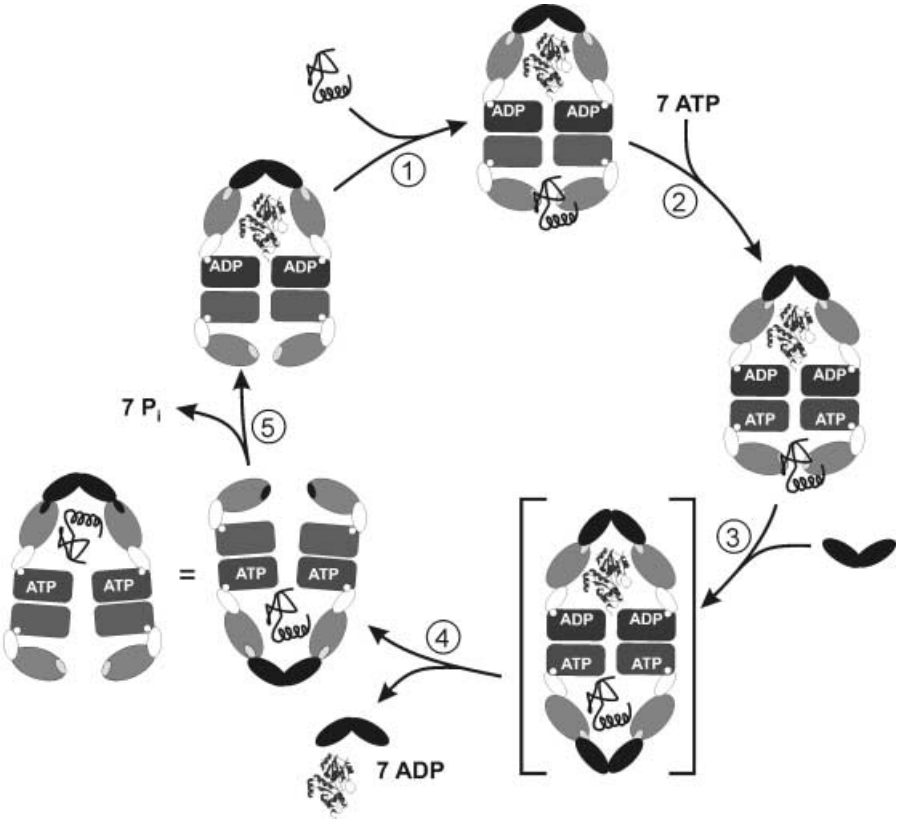

Figure 4. Model of the functional cycle of the GroE chaperone. Although GroEL is composed of two rings, the functional cycle is best described on the level of single rings, which represent the operational units of the chaperone. While both rings are active at the same time, they are in different phases of the cycle. Processing of an individual substrate polypeptide requires two revolutions of the GroE cycle during which the polypeptide remains associated with the same GroEL ring. For graphical reasons, the orientation of the GroE complex is reversed after step 4. The cycle of GroE-assisted folding can be dissected into three steps: capture, encapsulation/folding and release. During capture (1), a hydrophobic polypeptide is prevented from aggregation by binding to GroEL. The acceptor ring (bottom ring) is nucleotide free and therefore has a high affinity for the polypeptide. Binding of ATP (2) and GroES (3) to this ring induces a set of structural changes in GroEL. Most important, the affinity for the bound polypeptide is decreased, and it is released into the closed cavity where folding begins. Subsequent hydrolysis of ATP (5) induces a second conformational change in GroEL (top ring), which allows the bottom ring to bind polypeptide and initiate a new cycle. Upon binding of ATP and GroES in the next round, GroES is displaced from the top ring, and the substrate polypeptide is released (4). The formation of the symmetric complex shown in brackets is controversial.

state of GroE likely is the 'ADP bullet', in which the *trans* ring (i.e. the ring opposite of GroES) is in the high-affinity T form. Subsequent binding of ATP and GroES to this ring (fig. 4, steps 2 and 3) triggers a series of conformational changes in the GroEL molecule. As a result,

1) The affinity for both ADP and GroES in the *trans* ring decreases, causing the dissociation of both ligands (fig. 4, step 4) [64–66].
2) GroES covers the opening of the new *cis* cavity, thereby creating a closed compartment which sequesters the polypeptide substrate [33, 62].
3) The polypeptide binding site in the *cis* ring becomes buried within the cavity wall, causing the discharge of the polypeptide into the *cis* cavity.
4) The size of the cavity increases, giving the polypeptide sufficient room to undergo the structural rearrangements required for productive folding [23].

Once released from GroEL, the polypeptide will start to fold. Since it is still trapped in the central cavity, aggregation is no longer possible. In addition, the sterical restrictions resulting from confinement and the physical properties of the cavity wall may alter the energy landscape of the folding reaction [67, 68].

The formation of the symmetric intermediate shown in step 3 of the cycle is controversial. Though football-shaped GroE particles have been detected using electron microscopy (see section 2), it is not clear yet whether these molecules represent off-pathway products [69], or are indeed part of the chaperone cycle [70, 71]. The 'football' intermediate is an attractive concept since it would allow GroES to trap a 'new' polypeptide in the *cis* cavity, and at the same time trigger the release of the 'old' polypeptide from the *trans* ring.

Hydrolysis of the bound ATP (fig. 4, step 5), which takes ~10 s at 25 °C [55], represents the rate-limiting step in the

cycle and thus serves as a timer for encapsulation [58]. Once the ATP is hydrolyzed, the chaperone has completed its cycle and the next round starts, in which now the opposite ring will be charged with a polypeptide substrate. The release of the encapsulated protein occurs upon the subsequent binding of GroES/ATP (fig. 4, step 4).

At this stage, the ejected polypeptide is thought to undergo kinetic partitioning [64, 72]. Molecules that are not recognized by GroEL (native, committed or dead-end, see above) no longer participate in cycling. The remaining molecules may rebind and undergo another round of the GroE cycle, or bind to other molecular chaperones, or fold/assemble in bulk solution. The relative fractions of these species likely depend on the nature of the polypeptide as well as on the cellular context.

It is reasonable to assume that GroEL can process two substrates at a time, as shown in figure 4. According to this model, both rings (= operational units) are active, although they are in different phases of the chaperone cycle [58, 71]. As an example, the top ring after step 3 is loaded with a polypeptide that already has undergone folding in the cavity, and will become ejected in the next step. The polypeptide bound to the bottom ring, on the other hand, will be released into the cavity where folding is initiated. Thus, binding of GroES in step 3 has a dual function: it sequesters a 'new' polypeptide in the *cis* cavity, and it releases GroES and the processed polypeptide from the *trans* ring. In an alternative model, only one GroEL ring at a time is loaded with a polypeptide, whereas the second ring passes through the cycle in an empty state.

Owing to the limited volume of the cavity, GroE-mediated folding as shown in figure 4 is restricted to polypeptides smaller than ~ 60 kDa [73, 74]. Although larger proteins can bind to GroEL, they cannot become encapsulated underneath GroES. This is illustrated by the case of aconitase from yeast mitochondria, a monomeric enzyme of 82 kDa. Strains in which mt-hsp60, the yeast homologue of GroEL, was deleted were found to accumulate aggregated aconitase. Further analyses showed that both GroEL and GroES are required for the biogenesis of aconitase in vivo [75]. However, aconitase did not become encapsulated since GroES could only bind in *trans*, not in *cis* [76]. More important, GroES binding in *trans* was found to be essential for the release of GroEL-bound aconitase. It may thus reflect a general mechanism GroEL uses to dispose of substrates it cannot process [77].

GroE-mediated unfolding of proteins

A still open question is how GroE promotes the folding of proteins that are trapped in nonnative conformations. It is assumed that GroEL is capable of partially unfolding these proteins, thereby setting them back on the right track to the native state [78]. Several mechanisms have been suggested how GroEL accomplishes this task.

The most simple model, thermodynamic coupling [79], is based on the idea that for some folding intermediates structure formation and binding to GroEL may be antagonistic processes because they compete for the same (hydrophobic) residues. Since the amount of exposed hydrophobic surface generally decreases with the degree of folding, GroEL will preferentially bind to more unfolded conformations of a protein. Provided there is a rapid equilibrium between the various conformations of the polypeptide, GroEL will effectively unfold the protein by the law of mass action. This capability of GroEL has been demonstrated for a variety of relatively small proteins [79–82]. The coupling mechanism, however, has one important shortcoming. It would not allow a polypeptide to escape from a kinetic trap on its folding pathway, because, according to this model, all unfolding reactions occur in free solution at their intrinsic rates.

Based on experiments with Rubisco, it was also suggested that GroE can actively unfold a bound polypeptide [83]. According to this model, the movement of the apical domains, which occurs upon GroES binding (fig. 2C), may exert a mechanical stress on the bound polypeptide, thereby virtually tearing its structure apart. Importantly, this mechanism requires that the substrate be bound to multiple apical domains simultaneously [84]. Active unfolding, however, could yet not be observed with other stringent substrate proteins of GroE [45].

Perspectives

While our understanding of the basic mechanism(s) underlying GroE-assisted folding has increased considerably over the past years, there are still a number of issues that require further investigation. One of the most interesting problems that remains to be solved is the conformational changes a polypeptide undergoes when it is processed by the chaperone. This concerns unfolding reactions that occur upon binding of a polypeptide to GroEL as well as those unfolding events that may be associated with the subsequent binding of GroES (see above). Of equal importance is the question of what happens to the polypeptide while it folds in the cavity. Is the main purpose of sequestration to exclude other polypeptides and thus provide conditions of infinite dilution? Or does the cavity play a more active role, e.g. by lowering energy barriers on the folding pathway? Scientists have already begun to address these issues experimentally. The size of the GroE chaperone and the low conformational stability of the folding intermediates, however, make this a rather challenging undertaking.

Another issue concerns the general applicability of results obtained with a single 'model' substrate. Likely,

GroE provides a variety of 'tools' that promote the efficiency of protein folding, but not all of them may be observed when GroE processes a certain polypeptide substrate. This point is illustrated by the long-lasting debate about what is required to release a polypeptide from GroEL. In some cases, addition of ADP alone was found to be sufficient, in other cases ATP was required, while for a third set of protein substrates release was dependent on both ATP and GroES. These differences may simply reflect the heterogeneity of protein substrates GroE encounters in the cell. Thus, it will be crucial for our future understanding to identify the *E. coli* proteins that depend on GroE in their folding.

Acknowledgements. I thank Holger Grallert for help with preparation of figure 4. Financial support by the DFG is gratefully acknowledged.

1 Georgopoulos C., Hendrix R. W., Casjens S. R. and Kaiser A. D. (1973) Host participation in bacteriophage lambda head assembly. J. Mol. Biol. **76:** 45–60
2 Lorimer G. H. (2001) A personal account of chaperonin history. Plant Physiol. **125:** 38–41
3 Fayet O., Ziegelhoffer T. and Georgopoulos C. (1989) The groES and groEL heat shock gene products of *Escherichia coli* are essential for bacterial growth at all temperatures. J. Bact. **171:** 1379–1385
4 Goloubinoff P., Gatenby A. A. and Lorimer G. H. (1989) GroE heat-shock proteins promote assembly of foreign prokaryotic ribulose bisphoshate carboxylase oligomers in *Escherichia coli*. Nature **337:** 44–47
5 Goloubinoff P., Christeller J. T., Gatenby A. A. and Lorimer G. H. (1989) Reconstitution of active dimeric ribulose bisphosphate carboxylase from an unfolded state depends on two chaperonin proteins and Mg-ATP. Nature **342:** 884–889
6 Buchner J., Schmidt M., Fuchs M. Jaenicke R. Rudolph R., Schmid F. X. et al. (1991) GroE facilitates refolding of citrate synthase by suppressing aggregation. Biochemistry **30:** 1586–1591
7 Höll-Neugebauer B., Rudolph R., Schmidt M. and Buchner J. (1991) Reconstitution of a heat shock effect in vitro: influence of GroE on the thermal aggregation of alpha-glucosidase from yeast. Biochemistry **30:** 11609–11614
8 Fenton W. A. and Horwich A. L. (1997) GroEL-mediated protein folding. Protein Sci. **6:** 743–760
9 Sigler P. B., Xu Z., Rye H. S., Burston S. G., Fenton W. A. and Horwich A. L. (1998) Structure and function in GroEL-mediated protein folding. Annu. Rev. Biochem. **67:** 581–608
10 Thirumalai D. and Lorimer G. H. (2001) Chaperonin-mediated protein folding. Ann. Rev. Biophys. Biomol. Struct. **30:** 245–269
11 Grallert H. and Buchner J. (2001) A structural view of the GroE chaperone cycle. J. Struct. Biol. **135:** 95–103
12 Walter S. and Buchner J. (2002) Molecular chaperones – cellular machines for protein folding. Angew. Chem. Int. Ed. Engl. **41:** 1098–1113
13 Weber F., Keppel F., Georgopoulos C., Hayer-Hartl M. K. and Hartl F. U. (1998) The oligomeric structure of GroEL/GroES is required for biologically significant chaperonin function in protein folding. Nat. Struct. Biol. **5:** 977–985
14 Hendrix R. W. (1979) Purification and properties of groE, a host protein involved in bacteriophage assembly. J. Mol. Biol. **129:** 375–392
15 Saibil H. R., Dong Z., Wood S. and auf der Mauer A. (1991) Binding of chaperonins. Nature **353:** 25–26
16 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 accomodates the protein substrate within its central cavity. EMBO J. **11:** 4757–4765
17 Braig K., Simon M., Furuya F., Hainfeld J. F. and Horwich A. L. (1993) A polypeptide bound by the chaperonin groEL is localized within a central cavity. Proc. Natl. Acad. Sci. USA **90:** 3978–3982
18 Chen S., Roseman A. M., Hunter A. S., Wood S., Burston S. G., Ranson N. A. et al. (1994) Location of a folding protein and shape changes in GroEL-GroES complexes imaged by cryo-electron microscopy. Nature **371:** 261–264
19 Braig K., Otwinowski Z., Hedge R., Boisvert D. C., Joachimiak A., Horwich A. L. et al. (1994) The crystal structure of the bacterial chaperonin GroEL at 2.8 Å. Nature **371:** 578–586
20 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
21 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
22 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
23 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
24 Hunt J. F., Weaver A. J., Landry S. J., Gierasch L. M. and Deisenhofer J. (1996) The crystal structure of the GroES co-chaperonin at 2.8 Å resolution. Nature **379:** 37–45
25 Landry S. J., Zeilstra-Ryalls O., Fayet O., Georgopoulos C. and Gierasch L. M. (1993) Characterization of a functionally important mobile domain of GroES. Nature **364:** 255–258
26 Richardson A., Schwager F., Landry S. J. and Georgopoulos C. (2001) The importance of a mobile loop in regulating chaperonin/ co-chaperonin interaction: humans versus *Escherichia coli*. J. Biol. Chem. **276:** 4981–4987
27 Chandrasekhar G. N., Tilly K., Woolford C., Hendrix R. W. and Georgopoulos C. (1986) Purification and properties of the groES morphogenetic protein of *Escherichia coli*. J. Biol. Chem. **261:** 12414–12419
28 Jackson G. S., Staniforth R. A., Halsall D. J., Atkinson T., Holbrook J. J., Clarke A. R. et al. (1993) Binding and hydrolysis of nucleotides in the chaperonin catalytic cycle: Implications for the mechanism of assisted protein folding. Biochemistry **32:** 2554–2563
29 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
30 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
31 Azem A., Kessel M. and Goloubinoff P. (1994) Characterization of a functional $GroEL_{14}(GroES_7)_2$ chaperonin hetero-oligomer. Science **265:** 653–656
32 Schmidt M., Rutkat K., Rachel R., Pfeifer G., Jaenicke R., Viitanen P. V. et al. (1994) Symmetric complexes of GroE chaperonins as part of the functional cycle. Science **265:** 656–659
33 Weissman J. S., Hohl C. M., Kovalenko O., Kashi Y., Chen S., Braig K. et al. (1995) Mechanism of GroEL action: productive release of polypeptide from a sequestered position under GroES. Cell **83:** 577–587
34 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
35 Lorimer G. H. (1996) A quantitative assessment of the role of the chaperonin proteins in protein folding in vivo. FASEB J. **10:** 5–9

36 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
37 Lin Z., Schwarz F. P. and Eisenstein E. (1995) The hydrophobic nature of GroEL-substrate binding. J. Biol. Chem. **270:** 1011–1014
38 Katsumata K., Okazaki A. and Kuwajima K. (1996) Effect of GroEL on the re-folding kinetics of α-lactalbumin. J. Mol. Biol. **264:** 827–838
39 Aoki K., Taguchi H., Shindo Y., Yoshida M., Ogasahara K., Yutani K. et al. (1997) Calorimetric observation of a GroEL-protein binding reaction with little contribution of hydrophobic interaction. J. Biol. Chem. **272:** 32158–32162
40 Jaenicke R. and Seckler R. (1997) Protein misassembly in vitro. Adv. Protein Chem. **50:** 1–59
41 Fink A. L. (1998) Protein aggregation: folding aggregates, inclusion bodies and amyloid. Folding Des. **3:** R9–R23
42 Robinson C. V., Gross N., Eyles S. J., Ewbank J. J., Mayhew M., Hartl F. U. et al. (1994) Conformation of GroEL-bound α-lactalbumin probed by mass spectrometry. Nature **372:** 646–651
43 Lilie H. and Buchner J. (1995) Interaction of GroEL with a highly structured folding intermediate:Iterative binding cycles do not involve unfolding. Proc. Natl. Acad. Sci. USA **92:** 8100–8104
44 Goldberg M. S., Zhang J., Sondek S., Matthews C. R., Fox R. O. and Horwich A. L. (1997) Native-like structure of a protein-folding intermediate bound to the chaperonin GroEL. Proc. Natl. Acad. Sci. USA **94:** 1080–1085
45 Chen J., Walter S., Horwich A. L. and Smith D. L. (2001) Folding of malate dehydrogenase inside the GroEL-GroES cavity. Nat. Struct. Biol. **8:** 721–728
46 Landry S. J. and Gierasch L. (1991) The chaperonin GroEL binds a polypeptide in an a-helical conformation. Biochemistry **30:** 7359–7362
47 Schmidt M. and Buchner J. (1992) Interaction of GroE with an all-beta-protein. J. Biol. Chem. **267:** 16829–16833
48 Aoki K., Motojima F., Taguchi H., Yomo T. and Yoshida M. (2000) GroEL binds artificial proteins with random sequences. J. Biol. Chem. **275:** 13755–13758
49 Wang Z., Feng H., Landry S. J., Maxwell J. and Gierasch L. M. (1999) Basis of substrate binding by the chaperonin GroEL. Biochemistry **38:** 12537–12546
50 Falke S., Fisher M. T. and Gogol E. P. (2001) Structural changes in GroEL effected by binding a denatured protein substrate. J. Mol. Biol. **308:** 569–577
51 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
52 Gray T. E. and Fersht A. R. (1991) Cooperativity in ATP hydrolysis by GroEL is increased by GroES. FEBS Lett. **292:** 254–258
53 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
54 Yifrach O. and Horovitz A. (1994) Two lines of allosteric communication in the oligomeric chaperonin GroEL are revealed by the single mutation Arg196 $\rightarrow$ Ala. J. Mol. Biol. **243:** 397–401
55 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
56 Yifrach O. and Horovitz A. (1995) Nested cooperativity in the ATPase activity of the oligomeric chaperonin GroEL. Biochemistry **34:** 5303–5308
57 Horovitz A., Fridmann Y., Kafri G. and Yifrach O. (2001) Review: allostery in chaperonins. J. Struct. Biol. **135:** 104–114
58 Rye H. S., Roseman A. M., Chen S., Furtak K., Fenton W. A., Saibil H. R. et al. (1999) GroEL-GroES cycling: ATP and nonnative polypeptide direct alternation of folding-active rings. Cell **97:** 325–338
59 Ranson N. A., Burston S. G. and Clarke A. R. (1997) Binding, encapsulation and ejection: substrate dynamics during a chaperonin-assisted folding reaction. J. Mol. Biol. **266:** 656–664
60 Weissman J. S., Kashi Y., Fenton W. A. and Horwich A. L. (1994) GroEL-mediated protein folding proceeds by multiple rounds of binding and release of nonnative forms. Cell **78:** 693–702
61 Todd M. J., Lorimer G. H. and Thirumalai D. (1996) Chaperonin-facilitated protein folding: optimization of rate and yield by an iterative annealing mechanism. Proc. Natl. Acad. Sci. USA **93:** 4030–4035
62 Mayhew M., da Silva A. C., 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
63 Grallert H., Rutkat K. and Buchner J. (2000) Limits of protein folding inside GroE complexes. J. Biol. Chem. **275:** 20424–20430
64 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
65 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
66 Rye H. S., Burston S. G., Fenton W. A., Beechem J. M., Xu Z., Sigler P. B. et al. (1997) Distinct actions of cis and trans ATP within the double ring of the chaperonin GroEL. Nature **388:** 792–798
67 Betancourt M. R. and Thirumalai D. (1999) Exploring the kinetic requirements for enhancement of protein folding rates in the GroEL cavity. J. Mol. Biol. **287:** 627–644
68 Brinker A., Pfeifer G., Kerner M. J., Naylor D. J., Hartl F. U. and Hayer-Hartl M. K. (2001) Dual function of protein confinement in chaperonin-assisted protein folding. Cell **107:** 223–233
69 Hayer-Hartl M. K., Ewalt K. L. and Hartl F. U. (1999) On the role of symmetrical and asymmetrical chaperonin complexes in assisted protein folding. Biol. Chem. **380:** 531–540
70 Azem A., Diamant S., Kessel M., Weiss C. and Goloubinoff P. (1995) The protein-folding activity of chaperonins correlates with the symmetric $GroEL_{14}(GroES_7)_2$ heterooligomer. Proc. Natl. Acad. Sci. USA **92:** 12021X–12025
71 Sparrer H., Rutkat K. and Buchner J. (1997) Catalysis of protein folding by symmetric chaperone complexes. Proc. Natl. Acad. Sci. USA **94:** 1096–1100
72 Burston S. G., Weissman J. S., Farr G. W., Fenton W. A. and Horwich A. L. (1996) Release of both native and non-native proteins from a cis-only GroEL ternary complex. Nature **383:** 96–99
73 Ewalt K. L., Hendrick J. P., Houry W. A. and Hartl F. U. (1997) In vivo observation of polypeptide flux through the bacterial chaperonin system. Cell **90:** 491–500
74 Sakihawa C., Taguchi H., Makino Y. and Yoshida M. (1999) On the maximum size of proteins to stay and fold in the cavity of GroEL underneath GroES. J. Biol. Chem. **274:** 21251–21256
75 Dubaquie Y., Looser R., Fünfschilling U., Jeno P. and Rospert S. (1998) Identification of in vivo substrates of the yeast mitochondrial chaperonins reveals overlapping but non-identical requirement for hsp60 and hsp10. EMBO J. **15:** 5868–5876
76 Chaudhuri T. K., Farr G. W., Fenton W. A., Rospert S. and Horwich A. L. (2001) GroEL/GroES-mediated folding of a protein too large to be encapsulated. Cell **107:** 235–246
77 Sparrer H. and Buchner J. (1997) How GroES regulates binding of nonnative protein to GroEL. J. Biol. Chem. **272:** 14080–14086
78 Ranson N. A., Dunster N. J., Burston S. G. and Clarke A. R. (1995) Chaperonins can catalyse the reversal of early aggre-

gation steps when a protein misfolds. J. Mol. Biol. **250:** 581–586
79 Walter S., Lorimer G. H. and Schmid F. X. (1996) A thermodynamic coupling mechanism for GroEL-mediated unfolding. Proc. Natl. Acad. Sci. USA **93:** 9425–9430
80 Viitanen P. V., Donaldson G. K., Lorimer G. H., Lubben T. H. and Gatenby A. A. (1991) Complex interactions between the chaperonin 60 molecular chaperone and dihydrofolate reductase. Biochemistry **30:** 9716–9723
81 Zahn R., Spitzfaden C., Ottiger M., Wüthrich K. and Plückthun A. (1994) Destabilization of the complete protein secondary structure on binding to the chaperone GroEL. Nature **368:** 261–265
82 Zahn R., Perrett S., Stenberg G. and Fersht A. R. (1996) Catalysis of amide proton exchange by the molecular chaperones GroEL and SecB. Science **271:** 642–645
83 Shtilerman M., Lorimer G. H. and Englander S. W. (1999) Chaperonin function: folding by forced unfolding. Science **284:** 822–825
84 Farr G. W., Furtak K., Rowland M. B., Ranson N. A., Saibil H. R. and Horwich A. L. (2000) Multivalent binding of nonnative substrate proteins by the chaperonin GroEL. Cell **100:** 561–573
85 Boisvert D. C., Wang J., Otwinowski Z., Howich 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