Identification of a Major Inter-ring Coupling Step in the GroEL Reaction Cycle*

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Chaperonins are molecular chaperones that enhance the folding efficiency of substrate protein (1). They are generally classified on the basis of sequence comparison as members of one of the two families. The first family is represented by the group I chaperonins (e.g. GroEL from Escherichia coli) and is found in eubacteria as well as in organelles of eubacterial descent, such as mitochondria and plastids. The second chaperonin family is referred to as group II and comprises the chaperonins from Archaea (e.g. TF55/56 from Sulfolobus shibatae (2) and Thermosome from Pyrodictium occultum (3)) as well as from the eukaryotic cytosol (CCT from various organisms (4)). The group I and II chaperonins have the same domain organization within each subunit, namely an equatorial, ATP-binding domain that is linked by an intermediate hinge domain to an apical, substrate-binding domain (5). Moreover, all chaperonins have closely related quaternary arrangements. They are oligomers consisting of two seven-membered rings (GroEL), two eight-membered rings (CCT) and the thermosome), or two nine-membered rings (TF55/56) stacked back-to-back to form a double toroid cylinder that contains the two cavities encircled by each of the rings (5, 6).

No chaperonin has yet been isolated that is capable of progressing through its functional cycle without forming double-ring structures. There may be exceptions to the rule, although, the Thermoaerobacter brockii thermosome, which has initially been believed to be a genuine single-ring species (7), has been demonstrated to assemble into a double-ring structure during its functional cycle (8). Therefore, the Haloarcula marismortui thermosome, which assembles into a single-ring species variably comprising between 8 and 10 subunits (9), as well as the three single-ring mutants of GroEL (SR-T522I, SR-D115N, and SR-A399T), which have been shown to rescue GroEL-deficient E. coli (10), may well form double-ring structures in the presence of nucleotide. Hence, across the whole of biology, chaperonins are double-ring structures. The defining difference between the group I and II systems is that the former require a separate co-protein capable of capping the toroidal cavities, whereas the latter have 25-Å long helical protrusions emanating from the core of the apical domains that serve the same function (11).

The necessity for communication between the two rings has been highlighted by several studies on the GroE system, the most widely scrutinized of the chaperonins (12, 13). Protein folding in the presence of the whole GroE chaperonin system proceeds by a reaction cycle that comprises the binding of substrate protein, its encapsulation by GroES, a dwell period in the folding cavity and subsequent ejection into the bulk solution (14–18). Because each heptamer acts as a protein-binding unit, a full cycle is completed in the manner of a two-stroke machine and involves binding of substrate, nucleotide, as well as GroES to one ring of the complex followed by the same steps on the opposite ring (19–22). Inter-ring communication is of pivotal importance, as it mediates the structural asymmetry that imposes alternating functions on the two GroEL rings. Indeed, it has been shown that while positive cooperativity governs the binding of ATP, it is negative cooperativity that regulates the communication between rings (23). This led to the development of the nested model of cooperativity (24), whereby ATP binding within a ring follows a concerted system of the Monod-Wyman-Changeux model (25), and the negative cooperativity between rings follows the sequential Kosland-Nemethy-Filmer model (26).

To elucidate conformational rearrangements within and between rings induced by ATP binding and hydrolysis, we constructed a tryptophan-containing variant of GroEL by inserting a single tryptophan residue in position 485 in place of tyrosine (27), Y485W-GroEL. Residue 485 lies within the equatorial domain on the ring interface, on a loop that is connected by a helix to the ATP binding pocket and is well positioned to report intra-ring communication as well as the inter-ring communi-

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cition required for negative cooperativity. The response of this probe to nucleotide binding revealed a series of cooperative transitions in structure and showed how the system behaved when one or two rings were loaded with ATP or when the mixed-ring ADP-ATP complex was formed (27). In the work reported here we extend this study by probing structural rearrangements in a simplified system, namely a single-ring version of GroEL. Horwich and colleagues constructed this variant by introducing four mutations (R452E, E461A, S463A, and V464A), which delete the major contacts between the rings (16). Despite the apparently drastic procedure of halving the molecule, the functional properties of SR1 are remarkably similar to those of the parent GroEL structure: it is capable of sequestering unfolded proteins, binding GroES, and assisting the folding of substrates as successfully as the wild type (16). It cannot, however, eject GroES, and thus does not allow the substrate to undergo cycles of protein binding and release (16).

The fact that SR1 retains most of the essential properties of the parent structure prompted us to use the 485 probe in the context of the single ring to compare its behavior with that of wild-type GroEL. It was hoped that a comparison of the kinetic responses of the double- and single-ring structures (i.e. Y485W-GroEL and Y485W-SR1) would reveal the steps at which inter-ring coupling occurred in the whole molecule. The surprising finding is that only one step displays highly divergent thermodynamic properties in the SR1 species. The specificity of the effect allows us, at least in part, to deduce the mechanism of information transfer between the rings in the nucleotide-driven reaction cycle.

EXPERIMENTAL PROCEDURES

Standard Conditions

The standard reaction buffer in all experiments, except where stated otherwise, was 50 mM triethanolamine hydrochloride (pH 7.5), 50 mM KCl, and 20 mM MgCl2.

Proteins and Reagents

GroEL Tyr to Trp variants were constructed as described (28) and purified from E. coli strain TGO2 bearing the plasmid prc98a. The purification was undertaken by using the method described previously (22), except for the following two modifications. First, the Q-Sepharose column (50 mM Tris, pH 7.5) was followed by an additional Q-Sepharose column equilibrated either in 25% methanol, 50 mM BisTris (pH 6.0) in the case of Y485W-GroEL or 12% methanol, 50 mM BisTris (pH 6.0) in the case of Y485W-SR1. Second, the Q-Sepharose column was followed by an additional gel filtration step on a Sepharose-C200 column equilibrated in 100 mM NaN3, 50 mM Tris (pH 7.5). Analytical size-exclusion gel chromatography on a Superose-6 column was used to ascertain that there was no contamination of the single ring tryptophan variant with the parent structure. Concentrations of Y485W-GroEL and Y485W-SR1 were mixed, and aliquots were removed at various times over the course of the reaction. Each aliquot was quenched by addition of 10 volumes of 40 mM HCl containing 30 mg/ml acid-washed charcoal at the time of removal. The protein unfolds and releases the bound nucleotides as well as the products of the ATP hydrolysis. Any unhydrolyzed ATP and ADP bind to the charcoal leaving hydrolyzed SR1 in solution. After removal of charcoal (and uncleaved ATP) by centrifugation, the amount of 32P, in the supernatant was assayed by Cerenkov radiation counting. The background activity derived from spontaneously hydrolyzed phosphate in the original radioactive [γ-32P]ATP preparation was subtracted from all results. The Cerenkov counts were related to the amount of ATP hydrolyzed by measuring the radioactivity of an identical assay sample not treated with charcoal. This method measures the total product formed, whether it is free in solution or bound to the enzyme.

Stopped-flow Fluorimetry

All stopped-flow experiments were performed in the standard buffer using a thermostatted Applied Photophysics SX-17MV stopped-flow fluorimeter. Tryptophan-containing mutants were excited by monochromatic light at 295 nm and the resulting fluorescence was selected with a WG320 filter that cuts off all light below 320 nm. At least five fluorescence transients were averaged for any data point.

Data Analysis

All data fitting was carried out using Grafit 3.0 (Erithacus software). The dependence of the rates of reaction on the temperature was analyzed by plotting the natural logarithm of the rate (ln k) versus the inverse thermodynamic temperature (1/T) in classical Arrhenius fashion; the resultant line was fitted by linear regression. The slope m of the resultant straight line was then used to calculate the apparent energy of activation Ea according to the relation ln k = ln k0 - Ea/RT, where R = 8.314 J/moleK (33-35). The sigmoidal graphs generated by plotting the fluorescence amplitude versus the ATP concentration were fitted directly to the Hill equation to yield the equilibrium binding constant Kd (27). The dependence of the equilibrium dissociation constant Kd on the temperature was analyzed on the basis of the van't Hoff isotherm dln (Kd/RT) = -∆H/RT2, where R is the natural logarithm of the dissociation constant (ln Kd) was plotted against the inverse temperature (1/T) and fitted by linear regression. The slope m of the ensuing straight line fit is m = -∆H/RT (36). For all the linear fits in this study the correlation coefficient was greater than 0.99. The data of the single turnover measurement was fitted to a monoequilibrium function of the form f(t) = a1(1-exp{-kobs t}), where a1 represents the amplitude and signifies the total amount of ATP that has been hydrolyzed in the reaction, and kobs is the apparent rate constant. The data of the multiple turnover measurement was fitted to a straight line of the form f(t) = a kobs t, where the slope k is related to the steady state rate of ATP hydrolysis by m = kobs (19).

Site-directed Mutagenesis

SR1 was originally cloned in plc98a (Amersham Biosciences) expression vector. The SR1 clone was a kind gift from Arthur Horwich (Yale University). Mutagenesis was carried out by the “megaprimer” method of site-directed mutagenesis (37) in a PerkinElmer Life Sciences thermal cycler.

ATPase Activity Measurements

Linked ATPase Assay—ATP hydrolysis was measured using a coupled enzymatic assay as described previously (29, 30), except that the reaction mixture was left to equilibrate with all components apart from the chaperonin (either Y485W-GroEL or Y485W-SR1) to allow for the mopping up of unwanted ADP by pyruvate kinase. The reaction mixture contained ATP at a final concentration of 400 μM. The addition of chaperonin to a final concentration of 0.2 μM to the temperature-equilibrated reaction mixture initiated the measurement of the ATPase rate. The linking enzymes used were pyruvate kinase and lactate dehydrogenase from rabbit muscle (31, 32) and purchased from Sigma (P-0294). The rate of ATP hydrolysis was calculated as described (29).

The change in absorbance at 340 nm was measured in a thermostatted PerkinElmer Life Sciences Lambda-2 spectrophotometer.

Radioactive Orthophosphate Formation Assay—The assay for the hydrolysis of ATP by Y485W-SR1 was carried out under single turnover conditions (i.e. [SR1]initial << [ATP]), and under multiple turnover conditions (i.e. [SR1]initial >> [ATP]). Specifically, we used [SR1]initial = 140 μM = [ATP] for the single turnover, whereas for the multiple turnover we used [SR1]initial = 140 μM and [ATP] = 700 μM. These experiments were performed as described (19). Briefly, the required concentrations of nucleotide (containing about 10 μCi of [γ-32P]ATP) and Y485W-SR1 were mixed, and aliquots were removed at various times over the course of the reaction. Each aliquot was quenched by addition of 10 volumes of 40 mM HCl containing 30 mg/ml acid-washed charcoal at the time of removal. The protein unfolds and releases the bound nucleotides as well as the products of the ATP hydrolysis. Any unhydrolyzed ATP and ADP bind to the charcoal leaving hydrolyzed SR1 in solution. After removal of charcoal (and unhydrolyzed ATP) by centrifugation, the amount of 32P in the supernatant was assayed by Cerenkov radiation counting. The background activity derived from spontaneously hydrolyzed phosphate in the original radioactive [γ-32P]ATP preparation was subtracted from all results. The Cerenkov counts were related to the amount of ATP hydrolyzed by measuring the radioactivity of an identical assay sample not treated with charcoal. This method measures the total product formed, whether it is free in solution or bound to the enzyme.

The abbreviation used is: BisTris, 2-[bis(2-hydroxyethyl)aminol]-2-(hydroxymethyl)propane-1,3-diol.
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RESULTS

The observed rate constants for conformational shifts induced by ATP binding are the same across a wide temperature range; a rapid enhancement \((a)\), two easily resolved quench phases \((b\) and \(c)\), and finally, a slow enhancement \((d)\). Although this last phase has a rate constant of only \(0.76 \pm 0.03\) s\(^{-1}\), this value is still six times faster than the steady-state rate of hydrolysis in these conditions \((k_{cat} = 0.12\) s\(^{-1}\)), showing that these rearrangements precede the ATP-cleavage step. This behavior is strongly reminiscent of the four-phase behavior of the “wild-type,” double-ring structure when reported by the same optical signal \((27)\). To emphasize this close relationship the data for GroEL and SR1 are directly compared in Fig. 1b.

Despite this similarity of pattern, the fact that rate constants are roughly co-incident in two different systems does not demonstrate that the underlying physical processes are the same. However, Fig. 2 shows that the temperature responses of the readily measurable phases \((b-d)\) are identical. Such a result leads us to conclude that there is little difference in the prehydrolytic behavior of SR1 and GroEL. Hence, by these thermodynamic criteria, coupling between rings at this stage in the nucleotide-driven reaction cycle cannot be strong.

The above conclusion is strengthened by comparing the equilibrium binding affinities of ATP in the pre-hydrolytic phase. These can be measured by recording the fluorescence amplitude response as a function of ATP concentration on a time scale shorter than that of bond cleavage. Such a measurement is sensitive to the forward and reverse rates of all steps that precede hydrolysis and, although there is a small shift in \(K_i\) values between GroEL and SR1 (e.g. from 8.0 \((\pm 0.4)\) to 13.4 \((\pm 0.6)\) \(\mu M\) at 25 \(^\circ\)C), the temperature sensitivity is the same (see Fig. 3). In addition, the tightening of the interaction at high temperatures implies that the net binding energy of ATP arises from hydrophobic interactions consistent with displacement of water from non-polar surfaces.

Thermodynamic Properties of the Hydrolytic Step—When the rate of ATP hydrolysis is measured by orthodox steady-state kinetics, we see a profound divergence of behavior between the single- and double-ring structures (see Fig. 4). The Arrhenius activation enthalpy for GroEL \((94.2 \pm 3.3)\) kJ/mol) is more than twice that of SR1 \((42.0 \pm 0.7)\) kJ/mol). In view of the close similarity of all other measured parameters, this striking difference is worthy of attention. The most immediate issue is to question whether we are measuring the same step in the process of binding, rearrangement, bond cleavage, and product release in the steady-state SR1 cycle as we are in that of GroEL. In other words, does this radical change in the temperature dependence of the maximal rate signify a change in the nature of the rate-determining step? In the case of wild-type GroEL it has been shown that neither the dissociation of ADP and P\(_i\) nor the fluorescence-detected steps following binding of ATP limit the rate of the steady-state process \((19, 21)\). Fig. 5 shows this also to be the case for SR1; the first-order rate constant for the single-turnover reaction and the per-site steady-state rate of the multiple turnover reaction are the same, i.e. there is no rapid phase in the latter that reveals a mechanism in which the off-rate plays a part in the maximal rate of steady-state hydrolysis.

DISCUSSION

SR1 as a Paradigm for the Study of Ring-Ring Communication in GroEL—For SR1 to be useful in studying ring-ring communication it must retain structural and functional integrity. It has been shown previously that the Hill constant, which measures the degree of positive cooperativity within a GroEL ring, is hardly disturbed in SR1 showing that the communication between subunits within the heptamer is not strongly influenced by its contact with the other ring \((38)\). Second, the rate constants for conformational shifts induced by ATP binding are the same across a wide temperature range; there is thus
no change in thermodynamic parameters associated with the series of structural rearrangements following binding but preceding hydrolysis. We therefore conclude that halving the GroEL molecule has not led to a significant perturbation of either the structural or functional manifestations of inter-subunit contacts at the heptameric level of oligomeric organization. This view is further supported by Horwich and colleagues (17), who have demonstrated that SR1 remains a highly competent molecule because neither its ability to bind to GroES nor its capacity to sequester and refold substrate proteins appear to be impaired when compared with the wild type. All of the above reflects the crystallographic finding that inter-ring contacts are much less extensive than intra-ring contacts (6, 39). This view has most recently been underscored by electron microscopic studies on the effect of temperature on the size of the gap between the two rings of GroEL in the presence of ATP and an ATP-regenerating system. As the temperature is increased there is a concomitant increase in inter-ring spacing that is thought to reflect the weakening of inter-ring interactions (40, 41). By contrast, intra-ring interactions are comparatively uncompromised under these conditions, as demonstrated by the conservation of the structural integrity of the SR1 heptamer as well as of the individual rings in GroEL (42); neither heptamer dissociates into its component monomers.

It must be stated, however, that we do not assert that in the early steps of ATP-triggered rearrangements there are no differences between GroEL and SR1. It is clear that phase b (see Fig. 1) in the GroEL molecule shows a bi-sigmoidal dependence on ATP concentration depending on whether one ring in occupied by ATP at low nucleotide concentrations or both at high (38, 43). In the case of SR1 there is a simple uni-sigmoidal dependence as befits a single-ring system. Furthermore, in their most recent evaluation of F44W-GroEL, Amir and Horovitz (43) have identified an extra phase in the fluorescence response of this indole probe when they compare it to the behavior of its SR1 counterpart. This phase has a small amplitude (3% of the total quench) and reaches a maximal rate of 1 s\(^{-1}\) at saturating concentrations of ATP (43). The fact that it is absent in F44W-SR1 as well as in the R14G/A126V-variant of F44W-GroEL (which is defective in inter-ring negative cooperativity (44)) suggests it can be broadly ascribed to an early event in inter-ring signaling (see below). This is distinct from the coupling event we observe at the major rate-determining step in the ATPase cycle.

**Preservation of Asymmetry: a Reciprocating Structural Switch**—A kinetically unusual feature of GroEL is the aforementioned bi-sigmoidal behavior of the rate constant associated with phase b of the ATP-triggered conformational changes. This reflects the asymmetry of the two rings in so far as ligand binding to one ring causes a decrease in affinity for the same ligand in the opposite ring. However, although this represents negative cooperativity triggered upon ATP binding alone, the coupling is demonstrably weak, as the differential binding of ATP to the rings only affords a 6-fold difference in the kinetic half-saturation constant \(K_i\) to GroEL (from \(K_i = 113 (\pm 8) \mu M\) to \(K_i = 671 (\pm 15) \mu M\) (45) and from \(K_i = 122 (\pm 30) \mu M\) to \(K_i = 803 (\pm 18) \mu M\) (27)).

The most striking difference between SR1 and GroEL in the study described here is the huge increase in Arrhenius activation enthalpy of the rate-determining step in hydrolysis: the activation barrier is 52 kJ/mol higher in the double-ring structure. Given the relative lack of influence of contacts between rings on pre-hydrolytic steps triggered by nucleotide binding to the one ring (27, 38, 45), this divergence is likely to be mechanistically significant. We propose that this high barrier is because of the additional energy required to distort the second ring in the GroEL structure, i.e. it is a consequence of inter-ring communication. In the model shown in Fig. 6 we propose that the rate-limiting step in the hydrolysis of ATP is a conformational

![Graph](image-url)
of Y485W-GroEL, the data fit to the Hill equation with the following parameters: at 5 °C, 25 °C, and 45 °C, respectively. Data were collected at 5 °C, 25 °C, and 45 °C. All reactions were performed as described in the legend of Fig. 2. In the case of Y485W-SR1, the data fit to the Hill equation with the following parameters: at 5 °C, K°C = 19.7 (±0.8) μM and n°C = 2.6 (±0.3); at 25 °C, K°C = 8.0 (±0.4) μM and n°C = 2.6 (±0.4); at 45 °C, K°C = 4.8 (±0.2) μM and n°C = 2.5 (±0.3). In the case of Y485W-GroEL, the data fit to the Hill equation with the following parameters: at 5 °C, K°C = 28.8 (±1.2) μM and n°C = 2.2 (±0.4); at 25 °C, K°C = 13.4 (±0.6) μM and n°C = 2.5 (±0.3); at 45 °C, K°C = 7.1 (±0.3) μM and n°C = 2.6 (±0.4). Quoted errors are S.E. calculated from least-squares fits to the data. The error bars reflect the S.D. as calculated by the statistical law of error propagation through logarithmic functions.

The reaction is endothermic with respect to the association of ATP. The activation energy E\text{a}\, associated with the cleavage of ATP is calculated from the slope as 94.2 (±3.3) kJ/mol for the wild-type and as 42.0 (±0.7) kJ/mol for the SR1. In conclusion, the energy barrier to ATP hydrolysis is roughly twice as high for the Y485W-GroEL compared with that of Y485W-SR1 (E\text{a}\text{,GroEL}/E\text{a}\text{,SR1} = 2.2 (±0.1)). Quoted errors are S.E. calculated from least-squares fits to the data. The error bars reflect the S.D. as calculated by the statistical law of error propagation through logarithmic functions.

High Arrhenius barriers are associated with extensive structural rearrangements, so the very high value (94 kJ/mol) recorded for the rate-limiting step in the GroEL ATPase cycle is consistent with the above argument.

We suggest that this slow rearrangement step in GroEL consists of a concerted and reciprocal alteration in the structure of the two rings, mediated by the inter-ring contacts, before the hydrolysis of ATP. The loss of all inter-ring contact in the SR1 version of the molecule means that this coupling amount to less than 5% in each case. The temperature dependence of the ATP binding affinity of Y485W-SR1 and Y485W-GroEL. The enthalpy of the ATP-binding reaction for each protein has been extracted from van't Hoff plots (ln K° versus 1/T). The plots, which are both linear and monophasic, reflect an exothermic ligand-dissociation reaction with apparent enthalpies of ΔH° = −25.7 (±0.9) kJ/mol for the Y485W-GroEL and −25.6 (±1.2) kJ/mol for Y485W-SR1 (C), respectively. The reaction is endothermic with respect to the association of ATP. Therefore, the higher the temperature, the tighter ATP will bind to GroEL. All reactions were performed as described in the legend of Fig. 2. Quoted errors are S.E. calculated from least-squares fits to the data. The error bars reflect the S.D. as calculated by the statistical law of error propagation through logarithmic functions.
Fig. 5. Single turnover and multiple turnover of ATP by Y485W-SR1. The assay for the hydrolysis of ATP by Y485W-SR1 was carried out at 25 °C under standard conditions. Conditions for the single turnover were [SR1]_subunit = 140 μM = [ATP]. Conditions for the multiple turnover were [SR1]_subunit = 140 μM and [ATP] = 700 μM. The single turnover rate profile fits to a single exponential function with an observed rate constant of hydrolysis of \( k_{\text{obs}} = 0.103 \pm 0.006 \) s\(^{-1}\). The multiple turnover rate profile (inset, superimposed) is linear with a slope that corresponds to a maximum steady-state rate of hydrolysis of \( k_{\text{max}} \approx 0.093 \pm 0.002 \) s\(^{-1}\); it does not exhibit a burst phase. Within error, both rates are identical. In the inset, the two profiles are superimposed and show the linear trend to be the tangent to the single exponential curve in the point \( t = 0 \) (i.e., origin). The multiple turnover experiments track the transient formation of both enzyme-bound and free (released) products, so that a potential (pre-steady-state) burst phase of the magnitude of one turnover worth of products could be detected in addition to the subsequent steady-state rate process. Had the dissociation of ADP and P\(_i\), from the enzyme complex been rate-limiting, a burst phase with an amplitude of 140 μM P\(_i\) would have been observed under multiple turnover conditions. Also, the observed rate extracted from the single-exponential function would not have been identical to, but significantly higher than, the observed maximum steady-state rate hydrolysis constant (\( k_{\text{cat}} \approx 0.12 \) s\(^{-1}\)). Quoted errors are S.E. calculated from least-squares fits to the data. The compound errors of the Cerenkov counts and the initial rate calculations were less than 1% in all cases. Therefore, the error bars are smaller than the plot symbols.

effect is lost and a lower activation energy for this step is observed. This type of behavior is central to the maintenance of asymmetry in GroE. Once ATP is cleaved in the RT complex, the ADP product locks the first ring in the T-form, i.e., in the Koshland-Nemethy-Filmer part of the nested cooperativity model, the transition from RT to TR is achieved.

Additional support for such a mechanism comes from the behavior of ADP as an inhibitor of the ATPase of GroEL (20, 22, 27). If ADP is bound in trans to ATP, the hydrolysis of the latter is blocked. In terms of the model in Fig. 6, the presence of ADP in the opposite ring locks it in the T-form and prevents the conformational shift required for the hydrolytic step to occur. ATP hydrolysis thus serves as a rectifier in the reaction circuit that is the ATPase cycle of GroEL.

With respect to this explanation of the difference between GroEL and SR1, the aforementioned structural studies of Valpuesta and co-workers (40) are highly relevant. They employed transmission electron microscopy to monitor inter-ring spacings in GroEL at 25, 37, and 45 °C and found the ring-ring distance to increase concomitantly with temperature (40). The same study also found three other functional characteristics to be dependent on temperature; as the temperature is increased, inter-ring cooperativity is reduced, and substrate and GroES release are slowed down. These findings indicate that the single rings of GroEL behave in a more SR1-like manner as the temperature increases and have implications for the interpretation of temperature dependence in the studies described here.

In summary, we argue that ATP hydrolysis is directly associated with the major conformational inter-ring coupling step in the GroEL reaction cycle, i.e., the step at which structural rearrangements in one ring are communicated to the other is rate-limiting in the sequence of events in the overall reaction scheme. This slow passage of conformational information across the equatorial boundary from one ring to the other immediately prior to ATP cleavage is the molecular switching point for the preserving asymmetry in the RT to TR system.

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REFERENCES
1. Ellis, R. J. (1990) Semin. Cell Biol. 1, 1–9
2. Trent, J. D., Nimmesgern, E., Wall, J. S., Hartl, F. U., and Horwich, A. L. (1991) Nature 354, 490–493
3. Phipps, B. M., Hoffmann, A., Stetter, K. O., and Baumeister, W. (1991) EMBO J. 10, 1711–1722
4. Kubota, H., Hynes, G., and Willison, K. (1995) Gene (Amst.) 154, 231–236
5. Carrascosa, J. L., Llorca, O., and Valpuesta, J. M. (2001) Microsc. 32, 43–50
6. Sigler, P. B., Xu, Z., Rye, H. S., Burston, S. G., Fenton, W. A., and Horwich, A. L. (1998) Annu. Rev. Biochem. 67, 581–608
7. Truscott, K. N., Hoj, P. B., and Scopes, R. K. (1994) Eur. J. Biochem. 222, 277–284
8. Todd, M. J., Walke, S., Lorimer, G., Truscott, K., and Scopes, R. K. (1995) Biochemistry 34, 14932–14941
9. Franzetti, B., Schoch, G., Ebel, C., Gagnon, J., Ruigrok, R. W., and Zaccai, G. (2001) J. Biol. Chem. 276, 29906–29914
10. Sun, Z., Scott, D. J., and Lund, P. A. (2003) J. Mol. Biol. 332, 715–728
11. Klumpp, M., Baumeister, W., and Essen, L. O. (1997) Cell 91, 263–270
12. Weisman, J. S. (2001) Mol. Cell 7, 730–732
13. Walter, S. (2002) Cell Mol. Life Sci. 59, 1588–1597
14. Standfuss, R. A., Burston, S. G., Atkinson, T., and Clarke, A. R. (1994)
Allostery in the GroEL Chaperonin

15. Weissman, J. S., Kashi, Y., Fenton, W. A., and Horwich, A. L. (1994) Cell 78, 693–702
16. Weissman, J. S., Hohl, C. M., Kovalenko, O., Kashi, Y., Chen, S., Braig, K., Saibil, H. R., Fenton, W. A., and Horwich, A. L. (1995) Cell 82, 577–587
17. Weissman, J. S., Rye, H. S., Fenton, W. A., Beechem, J. M., and Horwich, A. L. (1996) Cell 84, 481–490
18. Ranson, N. M., Rye, H. S., Fenton, W. A., Beechem, J. M., and Horwich, A. L. (1996) J. Mol. Biol. 266, 656–664
19. Jackson, G. S., Staniforth, R. A., Halsall, D. J., Atkinson, T., Holbrook, J. J., Clarke, A. R., and Burston, S. G. (1997) J. Mol. Biol. 266, 577–587
20. Todd, M. J., Viitanen, P. V., and Lorimer, G. H. (1994) Science 265, 659–666
21. Burston, S. G., Ranson, N. A., and Clarke, A. R. (1995) J. Mol. Biol. 249, 138–152
22. Kad, N. M., Ranson, N. A., Cliff, M. J., and Clarke, A. R. (1998) J. Mol. Biol. 278, 267–278
23. Horovitz, A., Fridmann, Y., Kafri, G., and Yifrach, O. (2001) J. Struct. Biol. 135, 104–114
24. Yifrach, O., and Horovitz, A. (1995) Biochemistry 34, 5303–5308
25. Monod, J., Wyman, J., and Changeux, J. P. (1965) J. Mol. Biol. 12, 88–118
26. Koshland, D. E., Jr., Nemethy, G., and Filmer, D. (1966) Biochemistry 5, 365–385
27. Cliff, M. J., Kad, N. M., Hay, N., Lund, P. A., Webb, M. R., Burston, S. G., and Clarke, A. R. (1999) J. Mol. Biol. 293, 667–684
28. Gibbons, D. L., Hixson, J. D., Hay, N., Lund, P., Gorovits, B. M., Ybarra, J., and Horowitz, P. M. (1996) J. Biol. Chem. 271, 31989–31995
29. Kreuzer, K. N., and Jongsma, C. V. (1983) Methods Enzymol. 100, 144–160
30. Grallert, H., Rutkat, K., and Buchner, J. (2000) J. Biol. Chem. 275, 20424–20430
31. Finzi, A., Nyvold, S., and El Agroudi, M. (1992) J. Appl. Rabbit Res. 15, 745–750
32. Sutherland, G. B., Trapani, I. L., and Campbell, D. H. (1958) J. Appl. Physiol. 13, 367–372
33. Kohl, B., and Hofmann, K. P. (1987) Biophys. J. 52, 271–277
34. Cavichio, A., and Berber, M. M. (1979) Biochemistry 18, 1269–1275
35. Cornish-Bowden, A. (2002) J. Struct. Biol. 135, 104–114
36. Klausner, R. D., and Bertrand, S., and Muga, A. (2002) J. Biol. Chem. 277, 32587–32594
37. Sot, B., Galan, A., Carrascosa, J. L., Muga, A., and Valpuesta, J. M. (1998) Biochemistry 37, 7083–7088
38. Poso, D., Clarke, A. R., and Burston, S. G. (2004) J. Mol. Biol. 338, 969–977
39. Xu, Z., Horwich, A. L., and Sigler, P. B. (1997) Nature 388, 741–750
40. Llorca, O., Galan, A., Carrascosa, J. L., Muga, A., and Valpuesta, J. M. (1998) J. Biol. Chem. 273, 32587–32594
41. Sat, B., Galan, A., Valpuesta, J. M., Bertrand, S., and Muga, A. (2002) J. Biol. Chem. 277, 34024–34029
42. Galan, A., Llorca, O., Valpuesta, J. M., Perez-Perez, J., Carrascosa, J. L., Menendez, M., Banuelos, S., and Muga, A. (1999) Eur. J. Biochem. 259, 347–355
43. Amir, A., and Horovitz, A. (2004) J. Mol. Biol. 338, 979–988
44. Aharoni, A., and Horovitz, A. (1996) J. Mol. Biol. 238, 732–735
45. Yifrach, O., and Horovitz, A. (1998) Biochemistry 37, 7083–7088
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