Caught in the act: How ATP binding triggers cooperative conformational changes in a molecular machine

ARTICLE *in* MOLECULAR CELL · FEBRUARY 2002

Impact Factor: 14.02 · DOI: 10.1016/S1097-2765(02)00432-X · Source: PubMed

1 AUTHOR:

Lila M Gierasch
University of Massachusetts Amherst
233 PUBLICATIONS **11,528** CITATIONS

Selected Reading

Chauhan, D., Hideshima, T., Rosen, S., Reed, J.C., Kharbanda, S., and Anderson, K.C. (2001). J. Biol. Chem. *276*, 24453–24456.

Cheng, E.H.-Y., Wei, M.C., Weiler, S., Flavell, R.A., Mak, T.W., Lindsten, T., and Korsmeyer, S.J. (2001). Mol. Cell *8*, 705–711.

Ferri, K.F., Jacotot, E., Blanco, J., Esté, J.A., Zamzami, N., Susin, S.A., Xie, Z., Brothers, G., Reed, J.C., Penninger, J.M., and Kroemer, G. (2000). J. Exp. Med. *192*, 1081–1092.

Goldstein, J., Waterhouse, N., Juin, P., Evan, G., and Green, D. (2000). Nat. Cell Biol. *2*, 156–162.

Green, D.R., and Reed, J.C. (1998). Science *281*, 1309–1312.

Korsmeyer, S.J., Wei, M.C., Saito, M., Weiler, S., Oh, K.J., and Schlesinger, P.H. (2000). Cell Death Differ. *7*, 1166–1173.

Krajewska, M., Zapata, J.M., Meinhold-Heerlein, I., Hedayat, H., Monks, A., Shabaik, A., Bubendorf, L., Kallioniemi, O.-P., Kim, H., Reifenberger, G., et al. (2002). Neoplasia, in press.

Madesh, M., Antonsson, B., Srinivasula, S.M., Alnemri, E.S., and Hajnoczky, G. (2002). J. Biol. Chem., in press.

Martinou, J.-C., and Green, D.R. (2001). Nat. Rev. Mol. Cell. Biol. *2*, 63–67.

Schendel, S., Azimov, R., Pawlowski, K., Godzik, A., Kagan, B., and Reed, J.C. (1999). J. Biol. Chem. *274*, 21932–21936.

Scorrano, L., Ashiya, M., Buttle, K., Weiler, S., Oakes, S.A., Mannella, C.A., and Korsmeyer, S.J. (2002). Dev. Cell *2*, 55–67.

Vander Heiden, M.G., and Thompson, C.B. (1999). Nat. Cell Biol. *1*, E209–E216.

von Ahsen, O., Renken, C., Perkins, G., Kluck, R.M., Bossy-Wetzel, E., and Newmeyer, D.D. (2000). J. Cell Biol. *150*, 1027–1036.

Caught in the Act: How ATP Binding Triggers Cooperative Conformational Changes in a Molecular Machine

A paper recently published in *Cell* describes ATP-triggered conformational changes in the GroEL folding machine deciphered by use of cryo-electron microscopy, molecular engineering, and X-ray crystallographic data. Mechanistically crucial allosteric effects of ATP binding arise from rearrangement of interdomain electrostatic contacts.

In this era of molecular machines (Alberts, 1998), surprisingly few examples have been examined in adequate detail to understand their machine-like workings. The source of energy to drive most molecular machines derives from the nucleotide triphosphates ATP or GTP, yet we lack an understanding of the structural details by which this energy is converted into functional molecular rearrangements. In many cases, binding of ATP or GTP allosterically triggers domain movements at distances greater than 100 Å (Schnitzer, 2001). A major challenge is to relate the highly detailed information we obtain from static X-ray crystal structures to the dynamic steps involved in nucleotide-triggered domain rearrangements.

In the December 28, 2001 issue of *Cell*, the Saibil and Horwich laboratories (Ranson et al., 2001) describe synergistic use of cryo-electron microscopy, molecular engineering, and X-ray crystallographic data to decipher intimate molecular details of the response of GroEL to binding of ATP. Their work provides tantalizing structural hints about how ATP binding triggers the cycle of GroEL-facilitated protein folding. ATP binding has two distinct allosteric effects on GroEL function, as shown by extensive work from many laboratories (for reviews, see Sigler et al., 1998; Horovitz et al., 2001; Thirumalai and Lorimer, 2001). First, binding of ATP is positively cooperative within one ring of the two-ring tetradecameric structure. This intra-ring cooperativity depends upon subunit-subunit communication, which must be modulated by nucleotide binding. Secondly, binding of ATP to one ring shows negative cooperativity with the other ring, thus favoring an asymmetric overall conformation. This allosteric effect requires communication between the two rings of the chaperonin. These intra- and inter-ring allosteric effects are crucial to the mechanism of GroEL: Cooperative binding of ATP to a GroEL ring promotes subsequent binding of GroES to the same ring. When a substrate protein is present in the ring, ATP binding triggers its release into the encapsulated space underneath GroES, where productive folding takes place. At the same time, binding of ATP triggers events in the opposite ring—release of GroES, encapsulated substrate, and ADP. Subsequently, ATP hydrolysis in the folding-active "*cis*" ring cycles the chaperonin so that the opposite ring can accept ligands and become folding active. This set of coupled conformational changes creates a "two-stroke engine," with asymmetry between the two rings as a central feature, and relies on the influence of ATP binding both intra-ring and inter-ring. Examination of available crystal and cryo-EM structures points to large movements of the GroEL apical domains; these domains are circa 100 Å from the sites of ATP binding on the opposing ring, and therefore communication of conformational signals occurs over long distances.

GroEL in the absence of ligand is postulated to assume a TT allosteric state (subunits in both rings in the *tense* form, according to MWC nomenclature), using a "nested allosteric" model (Horovitz et al., 2001). ATP binding initially would convert the chaperonin to an RT state (subunits in the ATP-bound ring in the *relaxed* form) and then, at higher concentrations, to an RR state. ATP binding is thus expected to disrupt interactions that hold an empty ring in a T state, promoting a cooperative conformational rearrangement to an R state. Upon binding of multiple ATPs (Hill coefficient 2.5), the overall GroEL molecule should then be RT. It has heretofore been impossible to capture an image of this state and thus to understand how ATP binding initiates the cooperative intra-ring conformational change. The clever strategy of the new study is one that been successful in the past—use of a mutant GroEL (D398A, in this case) that is defective in ATP hydrolysis. This mutant binds ATP in a normal manner, sufficient to support substrate and GroES binding and productive folding, arguing that it is not structurally perturbed from the native chaperonin. Strikingly, cryo-EM images of GroEL(D398)-ATP indeed show distinct asymmetry between the two rings,

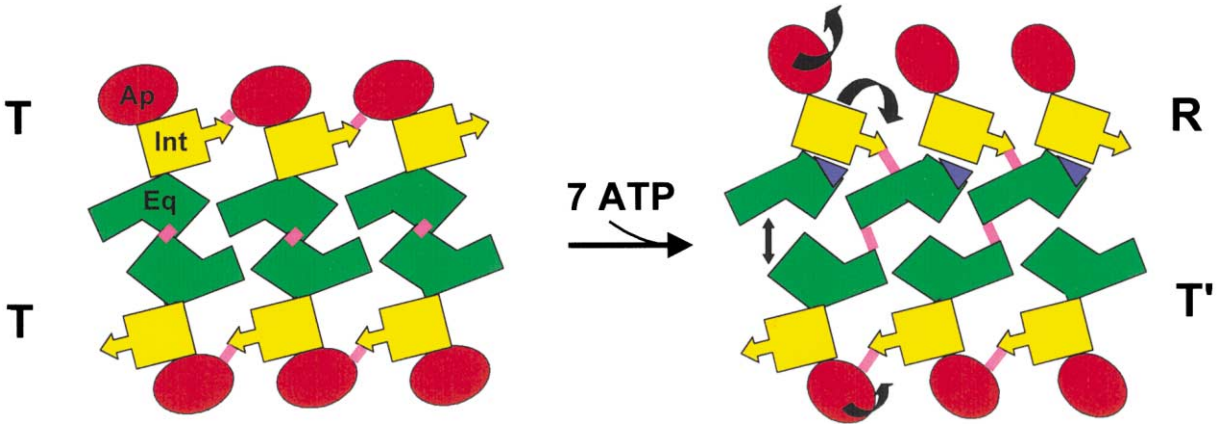

Figure 1. Schematic of the ATP-Mediated Intra- and Inter-Ring Subunit Rearrangements in GroEL

The allosteric effects of ATP binding to unliganded GroEL are illustrated in this schematic diagram. Apical domains (labeled "Ap" on one monomer) are shown in red, intermediate domains ("Int") in yellow, and equatorial domains ("Eq") in green. Only three monomers are shown in each ring in these side views, for clarity. Upon binding of ATP (blue triangles) to the upper ring, the intermediate domains undergo a 20° movement, disrupting the salt bridge between R197 and E386 on the apical domain of the adjacent monomer; a new contact forms with the equatorial domain of the same neighboring subunit. In the ATP-bound state, the inter-ring distance is increased, the apical domains of the ATP-bound ring rotate and tilt upwards, and the apical domains of the other ring move similarly but to a lesser extent. The interface between the rings is remodeled due to movements of the equatorial domains, such that some interactions are weakened and others strengthened. To help see these changes, the critical intra- and inter-ring electrostatic interactions are shown in pink, and the domain movements are indicated by black arrows.

as expected for an RT state. Modeling the cryo-EM images by fitting domain structures from X-ray crystal structures as rigid bodies allowed Ranson et al. to draw a plausible conclusion about which ring was ATP-bound, and hence in the "R" state. Analyzing its domain rearrangements led these authors to a detailed model for the nature of the ATP-mediated alterations in intersubunit interactions (Figure 1). Concerning the changes within a ring, the intermediate domain of the ATP-bound ring underwent a 20° downward tilt with respect to unliganded GroEL. (The intermediate domain is a hinge between the ATP binding equatorial domain and the peptide binding apical domains.) The downward tilt was accompanied by an upward tilt and anticlockwise twist of the apical domain and breakage of a salt bridge between E386 (on the intermediate domain) and R197 (on the apical domain of the adjacent subunit). Satisfyingly, the R197-E386 salt bridge had been implicated in cooperative allosteric conformational changes both in experimental kinetics studies (Yifrach and Horovitz, 1998) and in computer simulations (Ma and Karplus, 1998). In the R state, E386 appears to form a new salt bridge with a residue, K80, on the adjacent equatorial domain. Indeed, mutation of K80 weakens positive cooperativity of ATP binding. Thus, the new arrangement of salt bridges offers an explanation of how intra-ring cooperativity is mediated.

How are the conformational changes upon ATP binding communicated to the opposing ring? The cryo-EM images of the GroEL(D398A)-ATP complex show clear and pronounced widening of the interface between the rings relative to unliganded GroEL. The increased space between the rings suggests weakened inter-ring association and is consistent with earlier findings that ring exchange is facilitated in the presence of ATP (Burston et al., 1996). Along with the increase in separation between the rings, the equatorial domains of the R (ATP-bound) ring undergo reorientation upon ATP binding. Ranson et al. describe a consequent remodeling of the inter-ring interface that is consistent with the observed electron density and provides a mechanism for ring-ring communication. Specifically, they note that helix D of the equatorial domain, which leads from the ATP binding site to the inter-ring contact surface, appears to change its inter-ring contacts in response to ATP binding. This new model stands in contrast to an earlier proposal from the X-ray crystal structure of the GroEL-GroES-ADP complex that invoked mutual equatorial domain tilting of the two rings (Sigler et al., 1998).

One can ask whether the effects of ATP on otherwise unliganded GroEL reflect physiologically meaningful allosteric changes. By including the single-ring GroEL mutant, SR1, to trap any released GroES and prevent its rebinding, the authors were able to treat a stable GroEL-GroES-ADP complex briefly with ATP and rapidly freeze the sample. Any "bullet" complexes observed arose from the binding of ATP to the unliganded ring, allowing assessment of its effects on the GroES-GroEL complex. Intriguingly, the same rotation of apical domains (anticlockwise) was observed upon ATP binding to the open ring as in the GroEL(D398A) stand-alone structure, but the R197 to E386 salt bridge remained intact. Thus, the conversion from T to R state for the ATP-bound ring in this context appears to be incomplete, arguing for sequential steps in the action of ATP binding, as in a Koshland-Némethy-Filmer allosteric model (Koshland et al., 1966).

The increasing depth of understanding of the GroEL folding machine (for recent new insights see Brinker et al., 2001; Chaudhuri et al., 2001; as well as this study) have thrust it into a key role as a paradigm for many other classes of molecular machines, much like hemo-

globin has served as a paradigm for allosterically regulated ligand binding. Insights from GroEL will shed light on the way ATP energy can be harnessed to accomplish coordinated conformational changes, which in turn are exploited by oligomeric molecular machines for cellular functions as diverse as protein folding, vesicle trafficking, DNA replication, protein degradation, and protein translocation across membranes.

Lila M. Gierasch
Departments of Biochemistry
and Molecular Biology and Chemistry
University of Massachusetts
Amherst, Massachusetts 01003

Selected Reading

Alberts, B. (1998). Cell *92*, 291–294.

Brinker, A., Pfeifer, G., Kemer, M.J., Naylor, D.J., Hartl, F.-U., and Hayer-Hartl, M. (2001). Cell *107*, 223–233.

Burston, S.G., Weissman, J.S., Farr, G.W., Fenton, W.A., and Horwich, A.L. (1996). Nature *383*, 96–99.

Chaudhuri, T.K., Farr, G.W., Fenton, W.A., Rospert, S., and Horwich, A.L. (2001). Cell *107*, 235–246.

Horovitz, A., Fridmann, Y., Kafri, G., and Yifrach, O. (2001). J. Struct. Biol. *135*, 104–114.

Koshland, D.E., Jr., Némethy, G., and Filmer, D. (1966). Biochemistry *5*, 365–385.

Ma, J., and Karplus, M. (1998). Proc. Natl. Acad. Sci. USA *95*, 8502–8507.

Sigler, P., Xu, Z., Rye, H.S., Burston, S.G., Fenton, W.A., and Horwich, A.L. (1998). Annu. Rev. Biochem. *67*, 581–608.

Ranson, N.A., Farr, G.W., Roseman, A.M., Gowen, B., Fenton, W.A., Horwich, A.L., and Saibil, H.R. (2001). Cell *107*, 869–879.

Schnitzer, M.J. (2001). Nature *410*, 878–881.

Thirumalai, D., and Lorimer, G.H. (2001). Annu. Rev. Biophys. Biomol. Struct. *30*, 245–269.

Yifrach, O., and Horovitz, A. (1998). J. Am. Chem. Soc. *120*, 13262–13263.

Nucleolomics: An Inventory of the Nucleolus

In the January 8 issue of *Current Biology*, two papers from the Lamond and Mann laboratories describe the largest proteomics analysis to date of a cellular compartment, the nucleolus. As a byproduct of this tour de force, a novel nuclear compartment, the paraspeckles, was identified.

The nucleolus is arguably one of the most essential cellular organelles. First accurately described by Rudolph Wagner in 1835 as a "fleck" in follicles from sheep, its critical function as the site of ribosomal gene expression and ribosome assembly was established in the 1960s. Despite its long history as a prime object of study for cell and molecular biologists, many fundamental questions regarding the structure and function of the nucleolus are still unanswered. Where in the nucleolus are transcribing ribosomal genes localized and how are they organized? What other functions apart from ribosome biogenesis occur in the nucleolus? Does the nucleolus serve regulatory functions and if so, what are the molecular mechanisms? As if this were not enough, recent reports point to a role of the nucleolus in a wide range of cellular processes such as cell cycle control, growth control via tumor suppressors, and even aging. A milestone in the study of the nucleolus has now been reached by creating an inventory of proteins found in the nucleolus of human cells (Andersen et al., 2002; Fox et al., 2002). This catalog of nucleolar components will provide the basis for a renewed effort to understand both the structure and function of the nucleolus.

The approach taken by the Mann and Lamond laboratories to identify nucleolar proteins is deceivingly simple. Nucleoli were purified from cultured cells, and the protein composition was analyzed using nanoelectrospray tandem mass spectrometry. Not surprisingly, the mixture of proteins in the nucleolus fraction was highly complex, and state of the art bioinformatics approaches that identify proteins directly from the available genome sequence were required to efficiently analyze the spectrometry data. In this manner, 271 nucleolar proteins were identified (Andersen et al., 2002). Reassuringly, many of the well-known nucleolar proteins such as nucleolin, protein B23, and fibrillarin were detected, indicating that the starting material was indeed enriched in nucleoli. As expected, many of the detected proteins were nucleic acid binding proteins, RNA-dependent helicases, ATPases, and ribosomal proteins presumably involved in maturation of pre-rRNA and ribosome biogenesis. In addition, more than 80 novel proteins were found. To test whether these were contaminants or were true nucleolar components, 18 of them were fluorescently tagged and confirmed to localize to the nucleolus when expressed in mammalian cells. Therefore, it can be surmised that most of the novel proteins are indeed nucleolar and do not represent contaminations from other nuclear compartments or cellular organelles.

Several unexpected proteins turned up in the nucleolus. Amongst them were several translation factors and disfulfide isomerase, an endoplasmic reticulum localized protein involved in folding of newly synthesized proteins. While these might also be contaminants, the recent evidence for translation within the nucleus (Iborra et al., 2001) raises the more intriguing possibility that these factors are involved in protein synthesis in the nucleus and perhaps in the nucleolus. Other unexpected components of the nucleolus include keratin, an intermediate filament protein usually found in the cytoplasm, lamins, typically found at the nuclear envelope, and tubulin, the building block of microtubules. The presence of these structural proteins might point to the existence of a structural framework of the nucleolus, an idea supported by the recent identification of NO145 as a major