Protein chaperones and non-protein substrates: on substrate promiscuity of GroEL

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Chaperonins are a group of molecular chaperones that form large multi subunit structures and are found in all forms of life. Encoded by the *groEL* and *groES* genes, bacterial chaperonins are required for appropriate folding of many cellular proteins. A significant number of bacterial species are known to express multiple copies of chaperonin genes, possibly to confer redundancy of GroEL function in these species. It is also likely that the paralogous GroELs might be undergoing diversification of function as a consequence of gene duplication. We argue in this article that different chaperonins in an organism might be involved in distinct biochemical functions that remain to be discovered, some of which might be modulated by different oligomeric states of the chaperonins.

Keywords: Bacterial species, chaperonins, cellular proteins, substrate promiscuity.

HEAT shock proteins (HSPs) act as molecular chaperones, where they are involved in a wide range of functions such as assisting proteins to fold to their final conformation, guiding proteins during secretion and preventing protein aggregation that might occur due to stress[1,2]. HSPs have been classified according to their molecular weight, e.g. the 70 kDa HSP being commonly referred to as Hsp70. The basic biochemical function of HSPs is that of non-specific binding and release of polypeptide substrates, which is controlled by their ATPase action[1].

One of the best characterized families of HSPs is the Hsp60 family, which is also commonly referred to as the chaperonins. The chaperonins are characterized by large cylindrical assemblies that assist misfolded or unfolded substrate proteins to reach their native state by ATP-dependent cycles of binding and release[3]. The substrate proteins are encapsulated in the cavity of these cylinders to fold productively. Chaperonins are classified into two classes: group I, with the members present in the cytosol of prokaryotes and the endo-symbiotically related membrane-bound eukaryotic organelles such as mitochondria and chloroplast; and group II, with members present in eukaryotic cytosols and archaea. Group I chaperonins

require the assistance of co-chaperonin, Hsp10. Examples of group I chaperonins include GroEL from *Escherichia coli* and several eubacteria[4]. Co-chaperonin for GroEL is known as GroES or Cpn10. On the other hand, group II chaperonins possess a built-in lid for encapsulation and thus act independent of the co-chaperonin. CCT chaperonins and the well-studied thermosome from the archeal branch of life are members of this class. Whereas group I chaperonins form homo-tetradecameric complexes, the members of group II form hetero oligomers.

Structure and function of *E. coli* GroEL

Understanding of the biology of chaperonin function is dominated by studies on the GroEL–GroES system of *E. coli*. Since the discovery of chaperonin function, genetic, biochemical and structural studies on the *E. coli* GroEL–GroES system have led to the knowledge of various aspects of its function[1,4]. From these studies it has emerged that the primary function of GroEL is to act as an entity that prevents the undesirable consequences of intracellular protein aggregation, and help the substrate proteins to attain their native-like structure via GroES- and ATP-mediated cycles of binding and release.

GroEL possesses a three-domain architecture, in which the central region of the polypeptide, spanning amino acid residues 191–376, constitutes the apical domain that is rich in hydrophobic residues and binds the non-native substrates and GroES. The equatorial domain spanning two extremities of the GroEL polypeptide, i.e. residues 6–133 and 409–523, is responsible for the ATPase activity and the bulk of inter-subunit and inter-ring interactions. The hinge-forming intermediate domain spans two regions on the polypeptide, viz. residues 134–190 and 377–408, and connects the equatorial and apical domains. The conformational changes resulting from ATP binding and hydrolysis at the equatorial domain are transmitted to the apical domain via this region[5,6].

The critical aspect of the chaperoning ability of GroEL resides in its tetradecameric arrangement with two isologous heptameric rings, each ring enclosing a cavity for the substrate proteins to bind. The cavity, whose volumes range between 85,000 and 175,000 Å^3, is capped by GroES, forming a sequestered environment for the
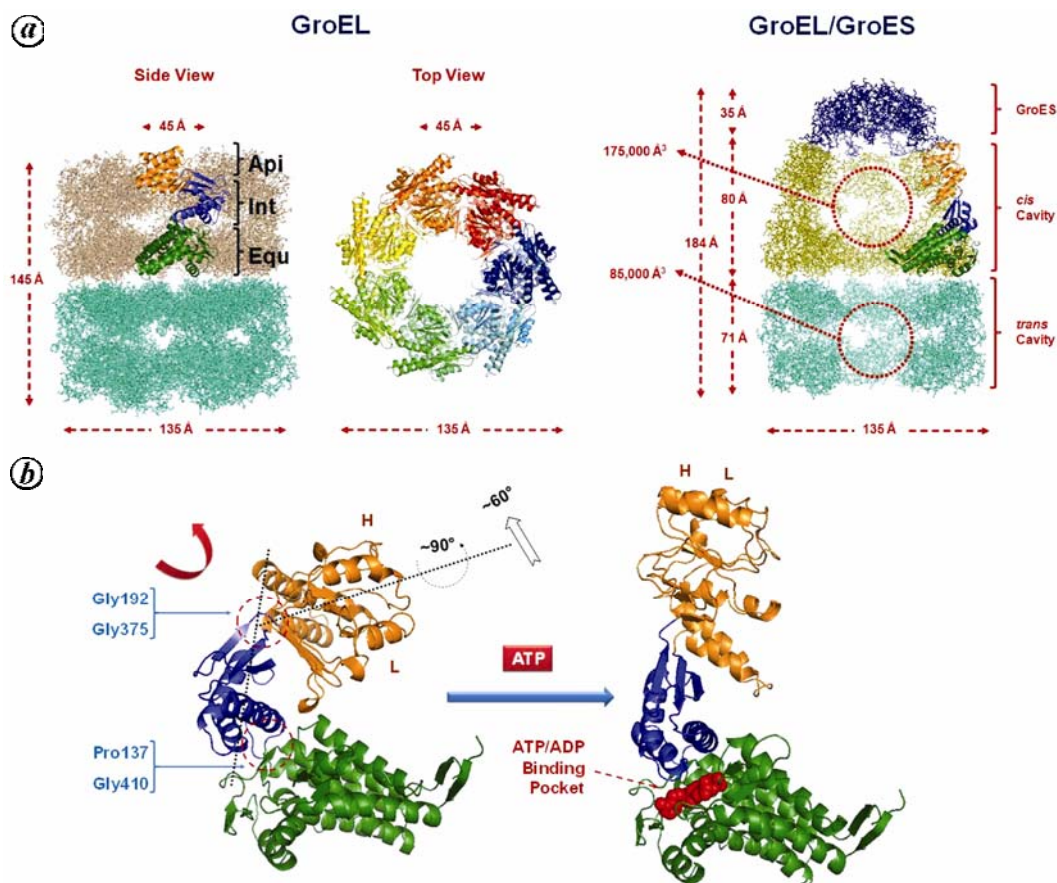
Figure 1. The architecture of *Escherichia coli* GroEL. *a*, Crystallographic models of *E. coli* GroEL and GroEL–GroES complex. Individual domains in one subunit of GroEL are indicated. Api, Apical domain; Int, Intermediate domain; Equ, Equatorial domain. GroES acting as a lid binds to GroEL asymmetrically, at the *cis* GroEL ring, wherein the substrate polypeptides are encapsulated. The other open ring is termed as the *trans* ring. Illustrations have been generated using Pymol 0.99, molecular visualization software from DeLano Scientific LLC, USA. Coordinates for the molecules were obtained from the structures deposited in the Protein Data Bank (PDB) with the ID: 1OEL for GroEL and 1AON for the GroEL–GroES complex structures. *b*, Crystallographic models of GroEL monomers depicting the domain motions upon ATP binding. GroEL in the substrate-bound form assumes a constricted conformation, positioning the substrates at the opening of the cavity. Upon ATP-binding, rotational movements in the intermediate domain and thereby upward movement of the apical domain result in extended conformation of the molecule. This releases the substrate into the cavity and exposes the helices H and L for GroES binding. Coordinates for the extended and constrained GroEL monomer were obtained from PDB: 1AON chain A and chain H, respectively.

substrate polypeptides to fold (Figure 1). GroEL interacts with a wide range of unfolded or partially folded proteins, and consequently is able to assist about 10–15% of the cellular proteins in *E. coli* to attain their native conformation. Thus, the major intracellular function of GroEL is understood to be that of a folding machine, without which cells would not survive.

Multiple copies of GroELs in bacteria

In view of its important participation in the protein-folding process, it is not surprising that GroEL occurs ubiquitously across species. The high sequence conservation among GroELs from different species is an indication that the mechanism of GroEL is universally conserved. Consequently, several chaperonin homologs from other bacteria have been shown to function in *E. coli*, suggesting that the spectra of substrate proteins in these bacteria must overlap considerably with those in *E. coli*[7,8]. As GroEL interacts with a wide range of substrate proteins, sequence analysis shows that the substrate-binding apical domain possesses significant plasticity in its sequence and structure[8]. On the other hand, the equatorial domain required for oligomerization exhibits better conservation. This observation has also been confirmed by random mutagenesis of GroELs, where the apical domain has been shown to be capable of absorbing large insertions or deletions, unlike the highly conserved equatorial domain[6]. Thus, by maintaining sufficient plasticity in its apical domain, GroEL appears poised to recognize a large range of substrates.

Availability of complete genome sequences of various bacteria has revealed the presence of multiple copies of

groEL genes, such as those in Actinobacteria, α-proteobacteria and Chlamydiae[7]. In these genomes, one (or more) of the multiple genes is arranged in an operon, with the cognate co-chaperonin *groES* being the first gene. The multiple *groEL* genes in different bacteria are likely to have arisen through either gene duplication in different lineages or horizontal gene transfer[7,8]. These evolutionary processes, therefore, have been believed to introduce new functional roles for the different copies of GroELs. For example, different GroELs in rhizobia have been shown to play different roles in nitrogen fixation, probably by encountering different substrates. Moreover, employing random mutagenesis to derive GroEL variants with improved ability to specifically fold GFP, was shown to result in variants with reduced ability to recognize their natural substrates and, consequently, these mutants turned out to be deficient in functioning as a general chaperone in the cell[9]. This study, therefore, elegantly demonstrated the contrast between the ability of GroEL in recognizing specific substrates on one hand, and a wide range of substrates on the other. Thus, although functional promiscuity in GroELs might be distributed to paralogous copies of GroELs, different substrates might exhibit characteristic affinities for interacting with GroEL. Such behaviour of protein–ligand interactions, termed as molecular promiscuity, has been widely observed in nature. Understanding the molecular basis of substrate promiscuity is important to appreciate its implications on the function of GroEL.

Molecular promiscuity in protein–ligand interactions

Structural features influencing macromolecular interactions are often governed by their topological determinants. We present here two examples of such interactions, which are the focus of this article, i.e. promiscuity of the chaperone functions.

MHC class I and CD1

The MHC molecules recognize peptides in their extended conformation by virtue of several contacts with the main chain of the substrate peptide. Such recognition allows MHC to bind peptides without any sequence specificity. However, in view of the substrate-binding groove being extended in nature, molecules with MHC-like fold also are able to bind non-peptides. For example, the human receptor CD1 and the MHC class I molecules share similar structural features in their ligand-binding grooves (Figure 2 *a*), where CD1 molecules have been shown to recognize foreign lipids and present them for specific recognition to the $CD4^-CD8^-\alpha\beta$ or $\gamma\delta TCR^+$ T-cells[10,11]. On the other hand, MHC molecules have been known to bind peptides and present them to the T-cell receptors[10].

These observations therefore indicate that proteins with similar structural features are capable of interacting with different macromolecular substrates, without any apparent specificity.

Janus chaperones

The promiscuity of functions has been observed in chaperones which exhibit dual specificity, viz. assist folding of RNA as well as protein substrates. One such family of proteins has been termed as Janus chaperones. These proteins are constituents of the 50S subunit of the *E. coli* ribosome and have been implicated in chaperoning the folding of 23S rRNA. Four of these, L15, L16, L18 and L19, have been shown to exhibit protein chaperone functions in addition to their RNA chaperone activity[12]. Although the RNA chaperone activity of these molecules has been shown in the intact ribosomes, their protein chaperone activity was demonstrated using purified proteins and *in vitro* folding assays. These belong to an emerging class of proteins, termed as intrinsically disordered proteins and are found on the periphery of the 50S ribosome (Figure 2 *b*). The protein chaperoning ability of these molecules has therefore been attributed to their disordered structure in isolation, and has been demonstrated to be as efficient as the well-known protein chaperone, Hsp90. Thus, these proteins exhibit pronounced protein as well as RNA chaperoning ability, confirming the underlying common structural principles of promiscuous functions.

Substrate promiscuity by GroEL – protein and non-protein substrates

Even as many studies point out broad substrate specificity of GroELs, these imply the same underlying biochemical mechanism, i.e. non-specific binding to the substrates, thereby preventing non-productive aggregation[4]. The non-specific binding is a consequence of recognition of substrates by the hydrophobic surfaces of GroEL, presented by the apical and equatorial domains. However, while recognizing a wide range of substrates, GroEL appears to have evolved the remarkable ability to bind only polypeptide substrates, distinctively differentiating these from other biopolymers. This therefore leads to an apparent paradox, i.e. how does GroEL distinguish between proteinaceous and non-protein substrates? It is reasonable to imagine that GroEL might encounter different macromolecules in the cell, but has evolved the capability to distinguish between the protein and non-protein substrates. Answering this question might require enhanced understanding of the possible biological roles of GroEL and the fundamental principles governing molecular recognition in general.

The ability to differentiate between partially folded polypeptides and other biopolymers might be conferred
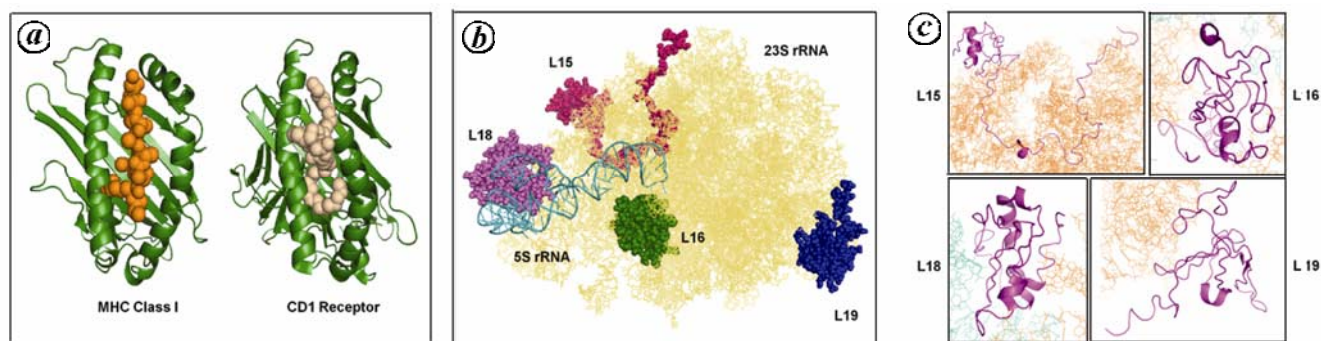
Figure 2. Examples of molecular promiscuity. *a*, Structural comparison of ligand-binding grooves on MHC class I and CD1 molecules. MHC class I molecules present foreign polypeptides to T-cells, whereas the CD1 receptor is implicated in presenting lipids to specific T-cells. Crystallographic models of the polypeptide bound to MHC class I molecule and the lipid moiety bound to CD1 receptor are presented in orange and white respectively. The coordinates for the MHC class I complex and CD1 complex were obtained from PDB: 1HSA and 2AKR respectively. *b*, Janus chaperones in prokaryotic ribosome: Crystallographic models of 50S ribosomal subunit showing the Janus chaperones L15, L16, L18 and L19, as indicated. *c*, Unstructured nature of the indicated individual chaperones presented in ribbon diagrams. The 23S rRNA is shown in yellow–orange and the 5S rRNA in cyan. Coordinates for the 50S ribosomal subunit and the individual chaperones were obtained from the RCSB protein databank ID: 3E1B.

upon GroEL by its specific conformational features. The interactions between GroEL and the substrates are principally hydrophobic, and therefore, exposure of hydrophobic surfaces of the apical and equatorial domains of GroEL by such conformational features is the key to substrate recognition. It has been shown that GroEL preferentially interacts with the well-conserved proteins of α/β domains, but with apparently no sequence similarity[13,14]. Therefore, the sequences of hydrophobic side chains of substrate polypeptides are not likely to participate in this specificity. Furthermore, employing mass spectrometry and NMR coupled with hydrogen-exchange techniques, it has been shown that GroEL in *E. coli* is inefficient in binding extended polypeptides, but is able to effectively bind the collapsed, molten, globule-like folding intermediates of the substrates[15–18]. Thus, discrimination among the substrates by GroEL might be such as to occlude substrates in extended conformation and selectively allow only globular substrates to bind.

With the ensuing arguments, the discrimination exhibited by GroEL among polymeric substrates appears to be due to the formation of central cavities by two back-to-back heptameric rings. It might therefore be likely that if GroEL were to be in a lower oligomeric form, its ability to differentiate between different biopolymers would cease to exist. The paralogous GroELs in different prokaryotic species might indeed exhibit such qualities and thereby be involved in distinct biochemical functions. Several chaperone proteins have been observed to exhibit such moonlighting in recognizing different macromolecules. Therefore, an understanding of this concept is essential to appreciate its effect on the promiscuity and function of GroEL.

Moonlighting in proteins

Moonlighting is the ability of a single polypeptide, not those which result from gene-fusion, splice variants or promiscuous enzymes, to perform two or more unrelated functions[19]. The discovery of increasing number of moonlighting proteins in recent years has added another dimension to the complexity of cellular networks. Although the discovery of moonlighting in eukaryotic proteins is well known, recent discoveries have demonstrated moonlighting in several prokaryotic proteins[20–22]. Different mechanisms have been proposed for moonlighting, including secretion into extracellular space, interactions with nucleic acids, changes in physico-chemical parameters such as temperature or redox condition of the cell, changes in oligomeric status or changes in the cellular concentration of ligands, substrates, co-factors or product[23].

Evolution of moonlighting might be a result of two fundamental cellular necessities. It has been proposed that moonlighting is principally exhibited by proteins which occur ubiquitously. This sounds plausible since many of the enzymes involved in the glycolytic pathway and citric acid cycle are ubiquitous and hence during the evolution an extra function might have been incorporated into these proteins. Examples include glyceraldehyde-3-phosphate dehydrogenase and aconitase (Figure 3). Moreover, as majority of proteins exhibit larger structure than that is necessary for performing a specific function, this provides an explanation for the origin of moonlighting. The apparent unused large surface areas exposed on the proteins might have evolved new pockets and active sites for performing novel functions. One protein performing several functions would therefore be advantageous for the cell in terms of energy conservation.

Moonlighting in GroEL

Several GroEL homologs have recently been demonstrated to exhibit moonlighting functions. As mentioned before, the mitochondrial Hsp60 has been shown to act as
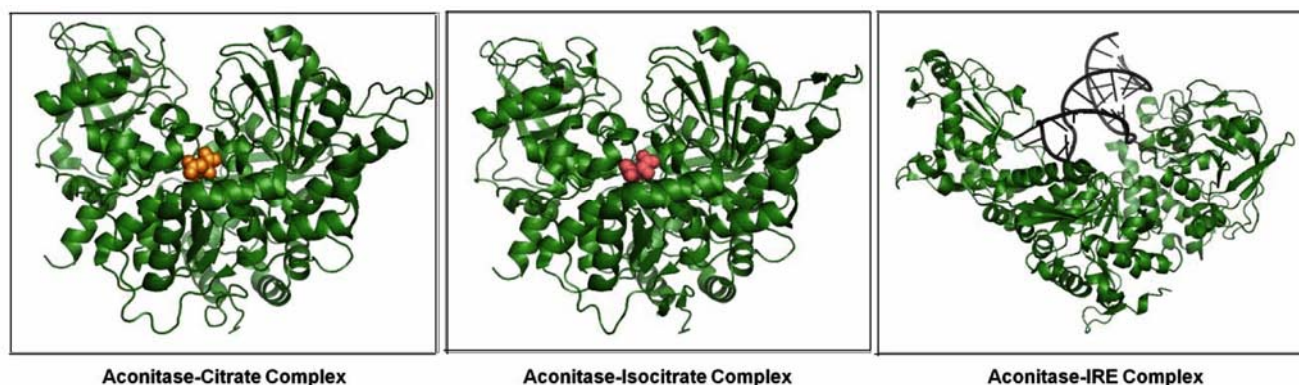
Figure 3. Aconitase as an example of moonlighting. Aconitase is a mitochondrial enzyme involved in catalysing the conversion of citrate to isocitrate in the citric acid cycle[29]. In response to iron depletion in the cell, the protein assumes a new conformation and converts its function to iron responsive element (IRE) binding protein. Aconitase in complex with citrate (1C96), isocitrate (1BOJ) and IRE element (2IPY) is presented. The conformational change in complex with IRE is apparent.

a protein chaperone *in vitro* and *in vivo*. On the other hand, the same chaperone has been demonstrated to bind single-stranded DNA *in vitro* and play a role in the stability and transmission of the nucleoid *in vivo*. Moreover, GroEL homologs from several insect symbionts such as *Enterobacter aerogenes* and *Xenorhabdus nematophila* have been shown to exhibit insect toxicity[24,25]. The toxicity of GroEL from *X. nematophila* has been demonstrated to be alleviated upon its interaction with the alpha-chitin. Mutational analysis followed by biochemical characterizations of the two homologs showed that the amino acid residues critical for toxicity are distinct from those essential for chaperone activity, suggesting that the two functions are independently operated.

Moonlighting by Mycobacterium tuberculosis GroEL1

The moonlighting functions of GroEL homologs might arise due to subtle alterations in substrate specificity as a consequence of differences in the oligomeric states of GroELs. Such an ability to differentiate between polypeptides and non-protein substrates by modulating their oligomeric properties appears promising to us from our recent studies on the paralogous GroELs of *M. tuberculosis*[6,26,27]. Biochemical and biophysical characterization of the recombinant *M. tuberculosis* GroELs has shown that despite possessing high sequence homology with *E. coli* GroEL, *M. tuberculosis* GroELs exhibit biochemical features that deviate significantly from that of *E. coli* GroEL. The most striking feature of *M. tuberculosis* GroELs is their oligomeric status, where these GroELs have been shown to exist as dimers, when purified from *E. coli*[26,28]. Moreover, these recombinant proteins displayed weak ATPase activity and GroES independence in preventing aggregation of the denatured polypeptides. *M. tuberculosis* GroEL1 was also demonstrated to associate with the nucleoids isolated from *M. tuberculosis* extracts[27]. Since the GroEL1 employed in this study was

purified from *E. coli*, it was presumably in the dimeric form that is involved in associating with nucleoids.

Apart from the suggestion that the two GroEL paralogs in *M. tuberculosis* perform different biochemical functions, another interesting finding has been that GroEL1 itself exists in different oligomeric forms: as a dimer, heptamer and tetradecamer[6]. Conversion between the heptamer and the tetradecamer was shown to be due to phosphorylation on a serine residue. It has thus been proposed that the naturally synthesized GroEL1 exists in an equilibrium between a dimer and a heptamer, and the heptamer to tetradecamer conversion is mediated by phosphorylation (Figure 4). Assuming that the tetradecameric form of GroEL1 might be an active chaperonin, and that it requires ATP for activity, it was therefore proposed that the phosphorylation event might act as an energy (ATP pool) conservation mechanism in slow-growing *M. tuberculosis*[6]. Moreover, similar multiple oligomeric forms of GroEL were observed in the mammalian mitochondria and plant chloroplasts, wherein they existed in the monomeric, single-ring heptameric and double-ring tetradecameric forms. Although mitochondrial GroEL was believed to operate as a single ring, the presence of the double-ring form and demonstration that the conversion of the single-ring form to the double-ring form is concentration and GroES-dependent, suggested that the double-ring form of GroEL might be required for its protein folding function[29,30]. Moreover, yeast mitochondrial GroELs were demonstrated to be associated with the stability and transmission of the nucleoid DNA[31]. Thus, by modulation of its oligomeric status, GroEL appears to switch between its different functional forms.

Nucleic acid binding by protein chaperones – possible functional implications

The observation of substrate spectrum of GroELs extending from polypeptides to polynucleotides raises an important
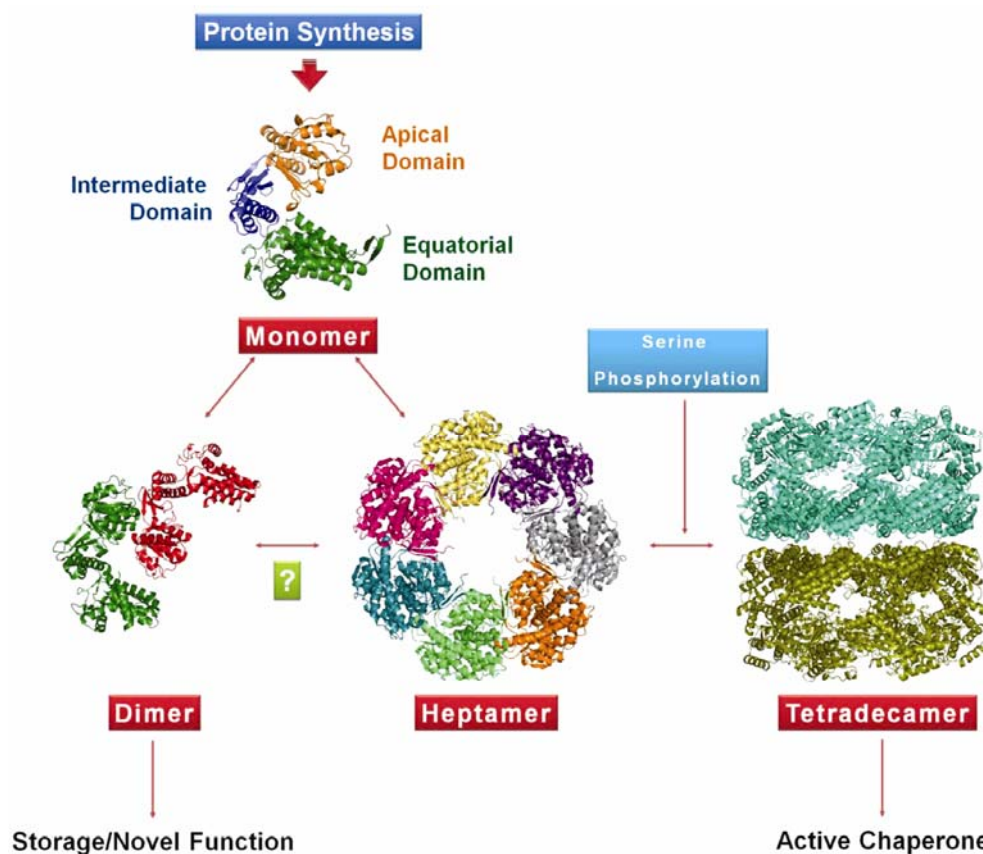
Figure 4. Model for the regulation of oligomerization in *Mycobacterium tuberculosis* GroEL1 mediated by phosphorylation. Upon synthesis, *M. tuberculosis* GroEL1 monomer might follow two pathways of oligomerization. The monomers either assemble into a dimer or a heptamer. The dimer might act as a storage form or might be involved in nucleoid formation. Function of the heptameric form is unknown. The signal for the probable conversion of the dimeric form into heptameric form is yet to be discerned (shown with a question mark). However, the signal for conversion of single-ring heptamer to double-ring tetradecamer is mediated by phosphorylation on serine residue(s). The tetradecameric GroEL is supposed to be the active form of the chaperonin.

question on whether such diversity of molecular recognition is also present in other HSPs as well. In this regard, recent observations on several chaperone molecules suggest that their interactome is not limited to proteins alone. For example, several protein chaperones in *Drosophila melanogastor* were demonstrated[23] to interact with nucleic acid, hnRNA. Studies have shown that the eukaryotic chaperones, Hsp110 and Hsp70, preferentially interact with AU-rich RNA species containing poly-U or AUUUA motifs[32,33]. Surprisingly, deletion mutation studies on these proteins showed that the RNA interaction is mediated by the nucleotide-binding domain, but not the substrate-binding domain[32,33], and that these interactions result in a highly dynamic complex with 1 : 1 stoichiometry of RNA to protein[34]. The yeast Hsp40 homologs, zuotin, was also demonstrated to interact with Z-DNA, tRNA and rRNA[35].

A few observations on prokaryotic chaperones also suggest their role in nucleic acid metabolism. The principal chaperones from *E. coli*, GroEL and DnaK, have been identified in bacterial degradosome[36–38] although direct

RNA-binding by these chaperones was not demonstrated. However, the co-chaperones DnaJ and GrpE have been demonstrated to exhibit modulator effects on the RNA-binding activity of DnaK in an *in vitro* reconstituted chaperone cycle[33]. Similarly, archaeal chaperonins were demonstrated to interact with 16S rRNA and help in its maturation[39]. Another small HSP from *E. coli* was shown to possess a novel RNA-binding domain and RNA-binding capacity[40–42].

Although the exact physiological role of the RNA chaperone is not known, with the current information on these interactions, it is believed that the protein chaperone and RNA chaperone functions might be resident in distinct molecular domains. Owing to the roles played by the molecular chaperones in maturation, stability and translation of mRNA, and the interaction of several co-chaperones with the tRNA and rRNA species, it is therefore believed that besides functioning as regular protein chaperones, these molecules might chaperone mRNA molecules and/or assist in the assembly of RNA–protein complexes and the export of RNA molecules[23,43].

Functional duality of *M. tuberculosis* GroEL

It is now important to address the structural features of GroELs that are likely to have evolved to confer substrate duality on them. Unlike the substrate-interacting apical domains, the equatorial domains in GroEL exhibit oligomerization-specific exposure. Whereas the apical domain remains exposed independent of the oligomerization state of GroEL, majority of the equatorial domain remains buried in the GroEL tetradecamer, but gets increasingly exposed as GroEL forms lower oligomers, such as a heptamer or a dimer. It might be possible that the GroEL1 dimer interacts with DNA via its nucleotide-binding equatorial domain. We therefore propose that the existence of the GroEL dimer in *M. tuberculosis* cytoplasm might be an evolutionarily-driven mechanism to address the two biochemically distinct functions attributed to GroEL, i.e. protein chaperone function and the nucleoid function. A comprehensive study might discern the understanding of the mechanism of conversion between the chaperone and nucleoid-associated functions of GroEL (Figure 3).

The available literature and our recent studies on *M. tuberculosis* GroELs lead us to the hypothesis that the ability to distinguish between proteins and other macromolecules is a consequence of the tetradecameric GroEL structure. We propose, based on the observation, that substrate recognition mechanisms in GroEL, driven by hydrophobic interactions, are incapable of differentiating between proteinaceous and non-protein substrates. Further, most of the macromolecules such as polynucleotides and polysachharides exist in an extended conformation, unlike the globular structure of proteins. On the other hand, polypeptides do not exist as extended polymers even under denaturing conditions. GroEL, thus appears to differentiate between extended and globular structures when it binds to substrates, rather than distinguishing the chemical characteristics of the substrates. Recognition of proteinaceous substrates therefore might be attributed to the formation of the cavity in GroEL, which might limit entry of the substrates only in partially folded conformation.

Conclusion

We propose that GroEL might interact with several substrates and that the preference to protein substrates is imparted by the formation of the central cavity. However, monomeric and dimeric molecules would not have such steric constraints and therefore could bind to any substrates. The presence of lower oligomeric forms therefore, might be implicated in recognizing hydrophobic substrates with extended conformations such as chitins in the case of GroEL from *X. nematophila*, and nucleic acids in the case of mitochondrial and mycobacterial GroEL homologs. The association of GroELs has been implicated in the maintenance of nucleoids, in the absence of general nucleoid-associated proteins[27]. Similarly, we propose that the promiscuous functions attributed to GroEL homologs might have been acquired during the course of evolution, to aid the organism in adapting to the ecological constrains, and that these functions might be modulated by the oligomeric status of the GroELs.

1. Hartl, F. U., Molecular chaperones in cellular protein folding. *Nature*, 1996, **381**, 571–579.
2. Hartl, F. U. and Hayer-Hartl, M., Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science*, 2003, **295**, 1852–1858.
3. Hartl, F. U. and Martin, J., Molecular chaperones in cellular protein folding. *Curr. Opin. Struct. Biol.*, 1995, **5**, 92–102.
4. Horwich, A. L., Fenton, W. A., Chapman, E. and Farr, G. W., Two families of chaperonin: physiology and mechanism. *Annu. Rev. Cell. Dev. Biol.*, 2007, **23**, 115–145.
5. Xu, Z., Horwich, A. L. and Sigler, P. B., The crystal structure of the asymmetric GroEL–GroES–(ADP)$_7$ chaperonin complex. *Nature*, 1997, **388**, 741–750.
6. Kumar, C. M. S., Khare, G., Srikanth, C. V., Tyagi, A. K., Sardesai, A. A. and Mande, S. C., Facilitated oligomerization of mycobacterial GroEL: evidence for phosphorylation-mediated oligomerization. *J. Bacteriol.*, 2009, **191**, 6525–6538.
7. Lund, P. A., Multiple chaperonins in bacteria – why so many? *FEMS Microbiol. Rev.*, 2009, **33**, 785–800.
8. Goyal, K., Qamra, R. and Mande, S. C., Multiple gene duplication and rapid evolution in the *groEL* gene: functional implications. *J. Mol. Evol.*, 2006, **63**, 781–787.
9. Wang, J., Herman, C., Tipton, K., Gross, C. and Weissman, J., Directed evolution of substrate-optimized GroEL/S chaperonins. *Cell*, 2002, **111**, 1027–1039.
10. Madden, D. R., Gorga, J. C., Strominger, J. L. and Wiley, D. C., The three-dimensional structure of HLA-B27 at 2.1 Å resolution suggests a general mechanism for tight peptide binding to MHC. *Cell*, 1992, **70**, 1035–1048.
11. Zajonc, D. M. *et al.*, Structural basis for CD1d presentation of a sulfatide derived from myelin and its implications for autoimmunity. *J. Exp. Med.*, 2005, **202**, 1517–1526.
12. Kovacs, D., Rakacs, M., Agoston, B., Lenkey, K., Semrad, K., Schroeder, R. and Tompa, P., Janus chaperones: assistance of both RNA- and protein-folding by ribosomal proteins. *FEBS Lett.*, 2009, **583**, 88–92.
13. Kerner, M. J. *et al.*, Proteome-wide analysis of chaperonin-dependent protein folding in *Escherichia coli*. *Cell*, 2005, **122**, 209–220.
14. Hirtreiter, A. M. *et al.*, Differential substrate specificity of group I and group II chaperonins in the archaeon *Methanosarcina mazei*. *Mol. Microbiol.*, 2009, **74**, 1152–1168.
15. Robinson, C. V. *et al.*, Conformation of GroEL-bound α-lactalbumin probed by mass spectrometry. *Nature*, 1995, **372**, 646–651.
16. Gervasoni, P., Staudenmann, W., James, P., Gehrig, P. and Plückthun, A., Beta-lactamase binds to GroEL in a conformation highly protected against hydrogen/deuterium exchange. *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 12189–12194.
17. Zahn, R., Spitzfaden, C., Ottiger, M., Wüthrich, K. and Plückthun, A., Destabilization of the complete protein secondary structure on binding to the chaperone GroEL. *Nature*, 1994, **368**, 261–265.
18. Goldberg, M. A., Zhang, J., Sondek, S., Matthews, C. R., Fox, R. O. and Horwich, A. L., Native-like structure of a protein-folding intermediate bound to the chaperonin GroEL. *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 1080–1085.

19. Jeffery, C. J., Molecular mechanisms for multitasking: recent crystal structures of moonlighting proteins, *Curr. Opin. Struct. Biol.*, 2004, **14**, 663–668.

20. Ostrovsky de Spicer, P. and Maloy, S., PutA protein, a membrane-associated flavin dehydrogenase, acts as a redox-dependent transcriptional regulator. *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 4295–4298.

21. Muro-Pastor, A. M., Ostrovsky, P. and Maloy, S., Regulation of gene expression by repressor localization: biochemical evidence that membrane and DNA binding by the PutA protein are mutually exclusive. *J. Bacteriol.*, 1997, **179**, 2788–2791.

22. Walden, W. E., Selezneva, A. I., Dupuy, J., Volbeda, A., Fontecilla-Camps, J. C., Theil, E. C. and Volz, K., Structure of dual function iron regulatory protein 1 complexed with ferritin IRE-RNA. *Science*, 2006, **314**, 1903–1908.

23. Jeffery, C. J., Moonlighting proteins – an update. *Mol. Biosyst.*, 2009, **5**, 345–350.

24. Yoshida, N. *et al.*, Protein function. Chaperonin turned insect toxin. *Nature*, 2001, **411**, 44.

25. Joshi, M. C. *et al.*, An insecticidal GroEL protein with chitin binding activity from *Xenorhabdus nematophila. J. Biol. Chem.*, 2008, **283**, 28287–28296.

26. Qamra, R., Srinivas, V. and Mande, S. C., *Mycobacterium tuberculosis* GroEL homologues unusually exist as lower oligomers and retain the ability to suppress aggregation of substrate proteins. *J. Mol. Biol.* 2004, **342**, 605–617.

27. Basu, D., Khare, G., Singh, S., Tyagi, A., Khosla, S. and Mande, S. C., A novel nucleoid-associated protein of *Mycobacterium tuberculosis* is a sequence homolog of GroEL. *Nucleic Acids Res.*, 2009, **37**, 4944–4954.

28. Qamra, R. and Mande, S. C., Crystal structure of the 65 kDa heat shock protein, chaperonin 60.2 of *Mycobacterium tuberculosis. J. Bacteriol.*, 2004, **186**, 8105–8113.

29. Dickson, R. *et al.*, Reconstitution of higher plant chloroplast chaperonin 60 tetradecamers active in protein folding. *J. Biol. Chem.*, 2000, **275**, 11829–11835.

30. Levy-Rimler, G. *et al.*, Type I chaperonins: not all are created equal. *Eur. J. Biochem.*, 2001, **268**, 3465–3472.

31. Kaufman, B. A., Kolesar, J. E., Perlman, P. S. and Butow, R. A., A function for the mitochondrial chaperonin Hsp60 in the structure and transmission of mitochondrial DNA nucleoids in *Saccharomyces cerevisiae. J. Cell Biol.*, 2003, **163**, 457–461.

32. Henics, T., Nagy, E., Oh, H. J., Csermely, P., Von Gabain, A. and Subjeck, J. R., Mammalian Hsp70 and Hsp110 proteins bind to RNA motifs involved in mRNA stability. *J. Biol. Chem.*, 1999, **274**, 17318–17324.

33. von Gabain, Z. A. and Henics, T., Analysis of sequence-specific binding of RNA to Hsp70 and its various homologues indicates the involvement of N- and C-terminal interactions. *RNA*, 2001, **7**, 1628–1637.

34. Wilson, G. M., Sutphen, K., Bolikal, S., Chuang, K. and Brewer, G., Thermodynamics and kinetics of Hsp70 association with A+U-rich mRNA-destabilizing sequences. *J. Biol. Chem.*, 2001, **276**, 44450–44456.

35. Wilhelm, M. L., Reinholt, J., Gangloff, J., Dirheimer, G. and Wilhelm, F. X., Transfer RNA binding protein in the nucleus of *Saccharomyces cerevisiae. FEBS Lett.*, 1994, **349**, 260–264.

36. Sohlberg, B., Lundberg, U., Hartl, F.-U. and Von Gabain, A., Functional interaction of heat shock protein GroEL with an RNase E-like activity in *Escherichia coli. Proc Natl. Acad. Sci. USA*, 1993, **90**, 277–281.

37. Georgellis, D., Sohlberg, B., Hartl, F.-U. and Von Gabain, A., Identification of GroEL as a constituent of an mRNA-protection complex in *Escherichia coli. Mol. Microbiol.*, 1995, **16**, 1259–1268.

38. Miczak, A., Kaberdin, V. R., Wei, C.-L. and Lin-Chao, S., Proteins associated with Rnase E in a multicomponent ribonucleolytic complex. *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 3865–3869.

39. Ruggero, D., Ciammaruconi, A. and Londei, P., The chaperonin of the archaeon *Sulfolobus solfataricus* is an RNA binding protein that participates in ribosomal RNA processing. *EMBO J.*, 1998, **17**, 3471–3477.

40. Korber, P., Stahl, J. M., Nierhaus, K. H. and Bardwell, J. C. A., Hsp15: a ribosome-associated heat shock protein. *EMBO J.*, 2000, **19**, 741–748.

41. Korber, P., Zander, T., Herschlag, D. and Bardwell, J. C. A., A new heat shock protein that binds nucleic acids. *J. Biol. Chem.*, 1999, **274**, 249–256.

42. Staker, B. L., Korber, P., Bardwell, J. C. A. and Saper, M. A., Structure of Hsp15 reveals a novel RNA-binding motif. *EMBO J.*, 2000, **19**, 749–757.

43. Henicsa, T. and Zimmera, C., RNA delivery by heat shock protein-70 into mammalian cells: a preliminary study. *Cell Biol. Int.*, 2009, **30**, 480–484.