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ARTICLE *in* PROTEINS STRUCTURE FUNCTION AND BIOINFORMATICS · SEPTEMBER 2002

Impact Factor: 2.63 · DOI: 10.1002/prot.10180 · Source: PubMed

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PROTEINS: Structure, Function, and Genetics 48:611–617 (2002)

Mapping Pathways of Allosteric Communication in GroEL by Analysis of Correlated Mutations

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ABSTRACT **An interesting example of an allosteric protein is the chaperonin GroEL. It undergoes adenosine 5′-triphosphate-induced conformational changes that are reflected in binding of adenosine 5′-triphosphate with positive cooperativity within rings and negative cooperativity between rings. Herein, correlated mutations in chaperonins are analyzed to unravel routes of allosteric communication in GroEL and in its complex with its co-chaperonin GroES. It is shown that analysis of correlated mutations in the chaperonin family can provide information about pathways of allosteric communication within GroEL and between GroEL and GroES. The results are discussed in the context of available structural, genetic, and biochemical data concerning short- and long-range interactions in the GroE system. Proteins 2002;48:611–617.**

Key words: nested allostery; cooperativity; GroES; chaperonins; molecular chaperones; protein folding

INTRODUCTION

Allosteric regulation of protein function is often achieved by conformational changes induced by binding of substrate or other ligand molecules. Such conformational changes are in many cases propagated to other parts of the protein that are distant in space from the ligand binding sites. A striking example of such an allosteric system is the chaperonin GroEL.[1,2] GroEL consists of two seven-member rings of identical subunits of 57.3 kDa, stacked back-to-back, with a cavity at each end. It has a helper-protein, GroES, that is a heptameric ring of identical subunits of 10 kDa.[3] GroEL undergoes adenosine 5′-triphosphate (ATP)-induced conformational changes[4] that are reflected in binding of ATP with positive cooperativity within rings and negative cooperativity between rings.[5] Each subunit of GroEL has three domains[6]:

1. an equatorial domain that contains an ATP binding site and forms all of the inter-ring contacts and many of the intra-ring contacts between subunits;
2. an apical domain that forms the opening of the central cavity and binds protein substrates and GroES; and
3. an intermediate domain that connects the apical and equatorial domains via two flexible hinge regions.

The crystal structure of the GroEL-GroES-$(ADP)_7$ complex shows that the (*cis*) ring with bound nucleotides and GroES undergoes a dramatic conformational change.[7] The intermediate domains swing down by 25° toward the equatorial domains and the apical domains undergo a 60° elevation and 90° clockwise twist. The only atomic resolution structure of an ATP-like state of GroEL (in the absence of GroES) is that of GroEL in complex with 14 molecules of ATPγS.[8] Little difference is found between this structure and that of unliganded GroEL[6] probably because of crystal packing forces, less than perfect mimicry of ATP by ATPγS, and/or the fact that both structures are of a double mutant with impaired negative inter-ring allostery.[9] Hence, the available information about ATP-induced conformational changes in GroEL is derived from electron cryo-microscopy, biochemical, and computational studies. These studies have shown that ATP binding breaks the Arg197-Glu386 salt bridge,[10] thus 1. allowing helix M in the intermediate domains to swing down and close off the ATP binding site in the equatorial domains[11] and 2. freeing the apical domains that rotate anti-clockwise.[12] ATP binding also leads to an increase in the separation between the two rings across the ring–ring interface.[13]

Although the structures of some stable allosteric states of GroEL are known,[4,6,7] the mechanism by which ligand-induced structural changes propagate through the molecule remains elusive (as in the case of most allosteric systems). Herein, we analyze correlated mutations in GroEL to unravel pathways of allosteric communication in this molecule and in its complex with GroES. Correlated mutations in proteins are thought to occur because there is greater selective pressure to maintain protein structure than sequence. Hence, a mutation that perturbs the structure at one site may be compensated for by mutations at other sites. Specific examples for the operation of such compensatory mechanisms are suppressor mutations revealed through genetic studies. In contrast, a way for large-scale identification of putative compensating partners is provided by statistical analysis of patterns of co-variations in amino acid sequences. It has often been assumed that compensatory mutations occur at sites near

Grant sponsors: Israel Science Foundation administered by The Israel Academy of Sciences and Humanities; MINERVA Foundation, Germany.

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Received 8 February 2002; Accepted 18 April 2002

the site of perturbation.[14] This has motivated the development of methods for detecting correlated mutations as a source for distance information in protein structure prediction.[15,16] Correlated mutations may, however, also occur at distant positions, thus, reflecting long-range energetic coupling in proteins.[17] Such energetic coupling is of particular importance in the case of allosteric proteins because of the interactions that exist between often distant ligand and effector binding sites. Herein, we demonstrate that analysis of correlated mutations in chaperonins can provide information about pathways of allosteric communication in this family of proteins.

METHODS

Sequence Database Construction

The non-redundant protein sequence database (as of September 2001) available on the NCBI BLAST server was searched for chaperonin sequences using PSI-BLAST.[18] Two sets of chaperonin sequences with a similarity to the sequence of GroEL that is greater than either 75 or 80% were constructed with GAP (from the CGC software package v10.2) using its default parameters and the BLOSUM62 amino acid substitution matrix.[19] Sequences that differed in their length from that of GroEL by more than 5% were removed. The sequence of GroEL[20] was corrected according to Horovitz et al.[21] The different chaperonin sequences were aligned with the GroEL sequence using five iterations of version 1.8 of the CLUSTAL W program.[22] Positions in which a gap was introduced in the GroEL sequence were eliminated. In addition, the first and last 10 positions in all the sequences were truncated because the multiple sequence alignment led to many gap insertions at these positions. This set of sequences was then analyzed for the presence of correlated mutations. Analysis of correlated mutations between cpn60 and cpn10 proteins was performed by constructing a set of cpn10 sequences from the same organisms and in an identical order as the set of cpn60 sequences.

Detection and Statistical Test for Correlated Mutations

Frequencies of all amino acids at all positions in the set of N sequences were calculated. The expected number of sequences, N_{EX}, that contain amino acid X at position i and amino acid Y at position j is given by $Nf_{X,i}f_{Y,j}$ where $f_{X,i}$ and $f_{Y,j}$ are the frequencies of X and Y at positions i and j, respectively. A difference between the observed number of sequences, N_{OBS}, that contain amino acid X at position i and amino acid Y at position j and N_{EX} may indicate that the two positions are coupled. The statistical significance of such differences is estimated using the χ^2 goodness-of-fit test, as follows:

$$\chi^2(i,j) = \sum_n (N_{n,OBS} - N_{n,EX})^2/N_{n,EX} \quad (1)$$

where n = kl is the number of different amino acid pairs that can be found at positions i and j given that k and l different kinds of amino acids are found at these two positions, respectively. The significance of the χ^2 values depends on the number of degrees of freedom, which is here equal to n − 1. A limitation of this approach is that no information is obtained about coupling between positions if one or both of them are fully conserved and, thus, have $\chi^2(i,j) = 0$. In addition, we disregard results for positions i,j if any $N_{n,EX}$ is less than one or if more than 20% of the $N_{n,EX}$ are less than five. The strength of our approach is that it does not involve assumptions about the chemical similarity of different amino acids[15,16] or that removal of a subset of sequences from the multiple sequence alignment represents a perturbation at only one site.[17] In addition, the probabilities of occurrence of different amino acids at different positions are calculated from the observed frequencies in the chaperonins without considering their frequencies in all proteins[17] because we are interested in correlations between pairs of positions in chaperonins and not in a measure of conservation.

RESULTS AND DISCUSSION

Analysis of correlated mutations in chaperonins was performed for two sets of sequences that have either more than 75% (72 sequences) or more than 80% (55 sequences) similarity to GroEL, respectively. Multiple sequence alignment was performed independently for each of the sets. Pairs of positions found to be with correlated mutations in one set of sequences may not be found in another set because addition or removal of sequences may affect amino acid conservation at a given position or lead to N_{EX} (see Methods) that is less than one or to more than 20% of the N_{EX} that are less than five. Pairs of positions found to be with correlated mutations that are statistically highly significant (confidence level >99.9%) in both sets are shown in Table I. Only 31 such pairs were found of a total of 87,571 [=N(N − 1)/2 where N equals 548 amino acids in GroEL minus 109 conserved positions and minus the 10 positions truncated at both the N and C termini] different possible pairs in the case of the set of sequences that have more than 75% similarity to GroEL. Thirteen of these pairs consist of one residue in the equatorial domain and one residue in the apical domain. Five pairs consist of one residue in the equatorial domain and one residue in the intermediate domain. Twelve pairs consist of residues that are both in the equatorial domain. Only one pair of residues consists of one residue in the intermediate domain and one residue in the apical domain.

It can be seen in Table I that different networks of coupled residues are found to exist in GroEL. The location in the GroEL structure[23] of some intra-subunit networks of coupled residues is shown in Figure 1. For example, Cys519 in the equatorial domain is coupled to Val158 in the intermediate domain which, in turn, is coupled to Ala356 in the apical domain (Fig. 1A). In this example, Cys519 and Ala356 are coupled only indirectly via Val158. Another network consists of Cys519 in the equatorial domain which is coupled to Val158 in the intermediate domain which in turn is coupled to Arg58 in the equatorial domain (Fig. 1A). In this example, Cys519 and Arg58 are also directly coupled to each other, thus forming a closed network. Networks that consist of more than three resi-

TABLE I. Pairs of Positions (i,j) in GroEL With Correlated Mutations Found to Be Statistically Significant in Both of Two Sets of Chaperonin 60 Sequences that Have Either >75% or >80% Similarity to GroEL, Respectively

Residue i[a]	Residue j[a]	Sequences with 75% similarity to GroEL (df; χ^2)[b]	Sequences with 80% similarity to GroEL (df; χ^2)[b]
D25 (E)	R58 (E)	3; 25.80	3; 34.41
D25 (E)	A81 (E)	3; 17.36	3; 32.09
D25 (E)	M267 (A)	5; 30.28	3; 32.59
D25 (E)	D523 (E)	3; 29.06	3; 30.03
K42 (E)	A81 (E)	3; 18.25	3; 33.55
K42 (E)	M267 (A)	5; 27.20	3; 30.24
K42 (E)	V323 (A)	3; 27.19	3; 34.61
K42 (E)	D328 (A)	3; 19.35	3; 24.96
R58 (E)	A81 (E)	3; 48.44	3; 31.55
R58 (E)	S135 (I)	3; 25.37	3; 16.49
R58 (E)	V158 (I)	3; 51.33	3; 34.99
R58 (E)	M267 (A)	5; 38.06	3; 25.12
R58 (E)	D328 (A)	3; 28.06	3; 20.93
R58 (E)	V442 (E)	5; 53.55	5; 35.10
R58 (E)	C519 (E)	3; 38.48	3; 23.95
R58 (E)	D523 (E)	3; 41.18	3; 34.43
A81 (E)	M267 (A)	5; 49.27	3; 43.80
A81 (E)	D328 (A)	3; 22.50	3; 17.63
A81 (E)	V442 (E)	5; 55.03	5; 40.29
A81 (E)	D523 (E)	3; 25.67	3; 19.75
S135 (I)	D523 (E)	3; 22.02	3; 17.93
V158 (I)	A356 (A)	5; 38.36	5; 21.21
V158 (I)	C519 (E)	3; 42.58	3; 31.40
V158 (I)	D523 (E)	3; 23.36	3; 16.88
M267 (A)	V442 (E)	8; 46.31	5; 37.95
M267 (A)	E518 (E)	5; 29.77	3; 30.31
M267 (A)	D523 (E)	5; 31.38	3; 19.98
D328 (A)	D523 (E)	3; 23.18	3; 17.38
A356 (A)	V442 (E)	8; 52.89	8; 33.03
D435 (E)	V442 (E)	5; 25.33	5; 20.55
V442 (E)	D523 (E)	5; 30.33	5; 22.82

[a]Residues are designated according to the sequence of GroEL. E, I, and A indicate whether a residue is located in the equatorial, intermediate, or apical domain of GroEL, respectively.
[b]df, degrees of freedom.

dues are also found. For example, Asp523 in the equatorial domain is coupled to Met267 in the apical domain which is coupled to Lys42 in the equatorial domain which, in turn, is coupled to Ala81 in the equatorial domain (Fig. 1B). Asp523 and Ala81 are also directly coupled to each other, thus forming a closed network of four residues.

Interestingly, little coupling between residues in GroES is observed (except for residues that are coupled indirectly via GroEL residues) perhaps because this protein does not appear to undergo ligand-induced conformational changes. Networks of residues in GroES coupled to residues in GroEL are, however, found to exist (Table II). The location in the structure of the GroEL–GroES complex[7] of some of these networks is shown in Figure 2. For example, the GroEL residues Arg58 in the equatorial domain and Val158 in the intermediate domain are coupled to each other and to Leu27 in the mobile loop of GroES, thus forming a closed network. Arg58 and Val158 in GroEL are also coupled to Tyr71 in GroES, thus forming another closed network. Arg58 is also coupled to Met267 in the apical domain of GroEL which, in turn, is coupled to Leu27 in the mobile loop of GroES.

In principle, coupling between residues may reflect interactions in the denatured state,[24] long-range electrostatic interactions,[25] or propagation of conformational changes. The fact that many of the coupled residues are not charged and that the networks involve residues in both GroEL and GroES (which do not interact with each other in the denatured state) suggests that in many cases the coupling here observed is attributable to conformational changes and that the networks, thus, reflect pathways of allosteric communication. In principle, such pathways may be within subunits, between subunits, or both. An interesting network of residues involved in intra-subunit interactions comprises residues Arg58, Asp328, and Ala81 (Table I). Simulations by Ma et al.[11] have shown that Arg58, Lys327, and Asp83 interact with each other in the ATP (R) state although they are not found to be in contact in the available crystal structures.[6–8] Remarkably, two unrelated theoretical methods, therefore, indicate that

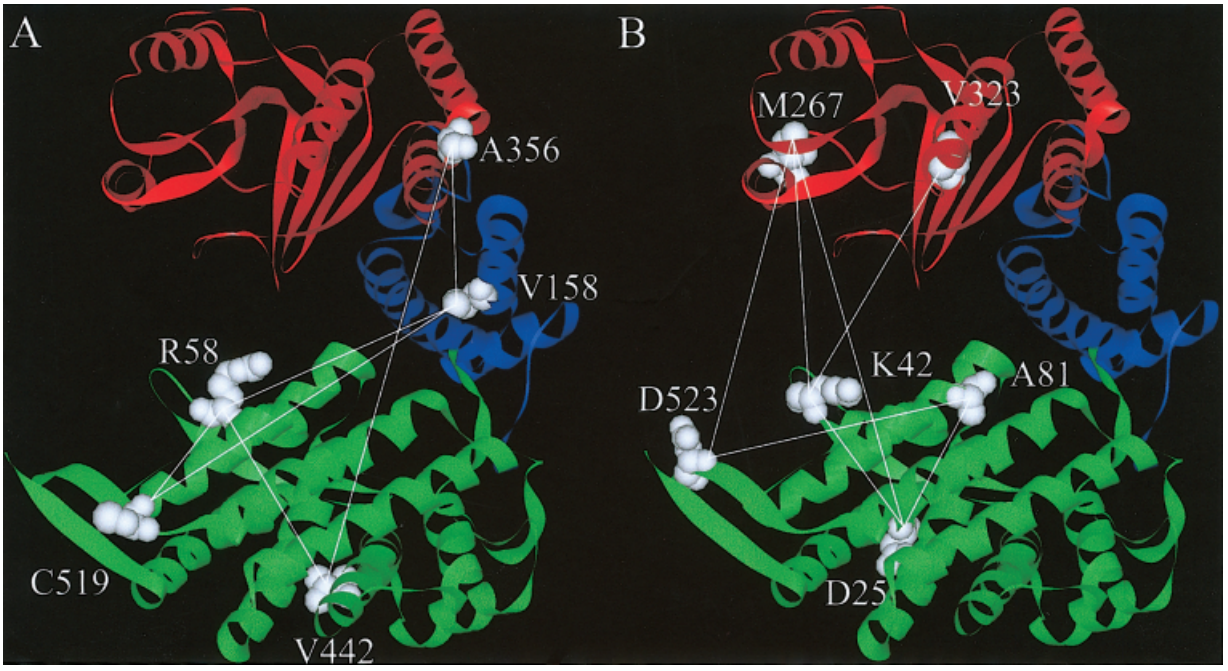

Fig. 1. Ribbon diagrams of the structure of a GroEL subunit (PDB code: 1OEL)[23] showing the locations of some intra-subunit networks of coupled residues (panels **A** and **B**). The equatorial, intermediate, and apical domains of GroEL are colored in green, blue, and red, respectively. Coupled residues are colored in white and designated using the single-letter code for amino acids. Coupling between residues is indicated by the white lines. The diagrams were drawn with WebLab ViewerLite 3.20 from Molecular Simulations.

TABLE II. Pairs of Positions in GroEL (i) and GroES (j) With Correlated Mutations Found to Be Statistically Significant in Both of Two Sets of Chaperonin 60 Sequences that Have Either >75% or >80% Similarity to GroEL, Respectively

Residue i in GroEL[a]	Residue j in GroES[b]	Sequences with 75% similarity to GroEL (df; χ^2)[c]	Sequences with 80% similarity to GroEL (df; χ^2)[c]
R58 (E)	S21	3; 35.27	3; 19.46
R58 (E)	L27	3; 32.71	3; 16.58
R58 (E)	S35	3; 43.87	3; 29.12
R58 (E)	Y71	5; 47.08	5; 32.73
A81 (E)	L27	3; 45.68	3; 33.16
A81 (E)	S35	3; 39.37	3; 25.30
A81 (E)	Y71	5; 44.53	5; 34.29
V158 (I)	S21	3; 30.12	3; 16.27
V158 (I)	L27	3; 33.52	3; 20.40
V158 (I)	S35	3; 32.90	3; 19.85
V158 (I)	Y71	5; 36.69	5; 24.77
M267 (A)	L27	5; 38.15	3; 31.06
M267 (A)	S35	5; 27.85	3; 16.85
D328 (A)	S21	3; 31.36	3; 34.05
D328 (A)	S35	3; 24.29	3; 21.49
D328 (A)	Y71	5; 28.11	5; 26.50
V442 (E)	L27	5; 42.79	5; 26.73
V442 (E)	S35	5; 39.34	5; 23.34

[a]Residues are designated according to the sequence of GroEL. E, I, and A indicate whether a residue is in the equatorial, intermediate, or apical domain of GroEL, respectively.
[b]Residues are designated according to the sequence of GroES.
[c]df, degrees of freedom.

Arg58 is coupled to residues at or nearby positions 327 and 83 with which it is not in contact according to the available crystal structures. It has been suggested that this network contributes to the coupled motion of the apical and equatorial domains.[11] An example that most likely reflects an intra-ring inter-subunit interaction is the indirect coupling between Lys42 and Cys519 via Arg58 and Ala81. Asp41 is an almost conserved residue (and thus we cannot detect its coupling to other residues) that makes an inter-subunit hydrogen bond with Thr522.[6] Lys42 is close

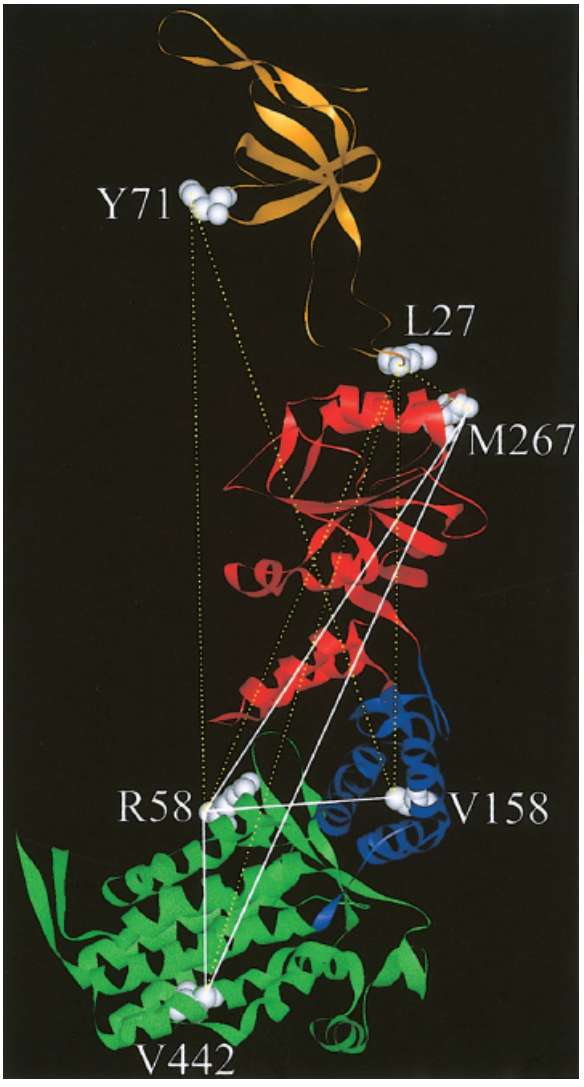

Fig. 2. Ribbon diagram of the structure of the complex of a GroEL subunit with a GroES subunit (PDB code: 1AON)[7] showing the location of some networks of coupled residues between the two proteins. GroES and the equatorial, intermediate, and apical domains of GroEL are colored in orange, green, blue, and red, respectively. Coupled residues are colored in white and designated using the single-letter code for amino acids. Coupling between residues in GroEL and GroES is indicated by the broken yellow lines. Coupling between residues in GroEL is indicated by the white lines. The diagram was drawn with WebLab ViewerLite 3.20 from Molecular Simulations.

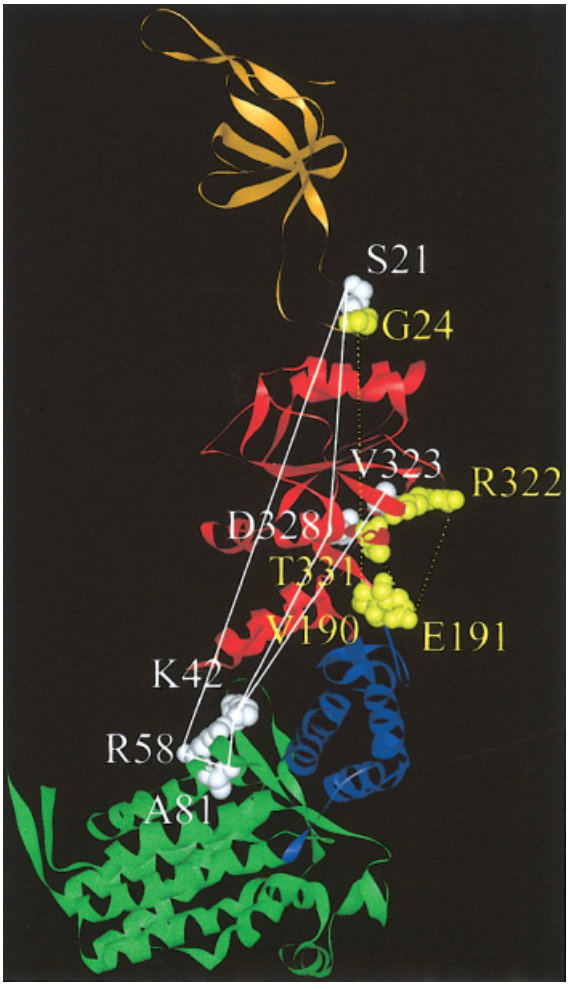

Fig. 3. Ribbon diagram of the structure of the complex of a GroEL subunit with a GroES subunit (PDB code: 1AON)[7] showing the location of networks of coupled residues between the two proteins revealed by genetic analysis and correlated mutations. GroES and the equatorial, intermediate, and apical domains of GroEL are colored in orange, green, blue, and red, respectively. Coupling between residues in yellow was revealed by genetic analysis[31] and is indicated by the dotted yellow lines. Coupling between residues in white is revealed by the correlated mutations analysis and is indicated by the white lines. Residues are designated using the single-letter code for amino acids. The diagram was drawn with WebLab ViewerLite 3.20 from Molecular Simulations.

to Asp41 and Cys519 is close to Thr522. In addition, Lys42 is also coupled indirectly via the apical domain residue Met267 to Asp523 which is also close to Thr522. In a previous study,[26] correlated mutations were found at positions 138 and 519 in GroEL. Coupling between these two positions was confirmed in that study by the finding that addition of ADP leads to dissociation of the Cys519 → Ser GroEL mutant but not the Cys138 → Ser, Cys519 → Ser double mutant. Here, correlated mutations at positions 519 and 138 are not observed because the latter position (and also the nearby positions 137, 139, and 140) is almost conserved in the set of sequences we analyzed. Correlated mutations at positions 519 and 135 (which is near to 138) are, however, observed in this study (Table I) and they most likely reflect long-range intra-subunit coupling. Another interesting example for a long-range intra-subunit interaction is the coupling between Asp25 in the equatorial domain and Met267 in the apical domain (Fig. 1B). This coupling may explain why the mutation Asp25 → Arg leads to impaired assisted folding[27] because Met267 is near the GroES binding site (see below). Long-range coupling is also found between Val442 in the equatorial domain and the apical domain residues Met267 and Ala356. Val442 is also coupled to Asp435 and both are near the ring–ring interface. The Cα–Cα distance between Val442 in the equatorial domain of one ring and Asp435 in the equatorial domain of the other ring is approximately 14 Å. Taken together, these observations may reflect a pathway

of information transfer from the ring–ring interface to the apical domains.

The interaction between GroEL and GroES is mediated by the mobile loop of GroES which spans residues Glu16 to Ala33. This polypeptide segment which is unstructured in uncomplexed GroES in solution[28] folds into a β-hairpin upon binding to GroEL.[29] The strength of our approach is reflected in the fact that three of the four residues in GroES found to be coupled to residues in GroEL are in (Ser21 and Leu27) or adjacent (Ser35) to the mobile loop. Ser21 and Leu27 are also in direct contact with each other.[28] All the other mobile loop residues except for Ala22 and Ile25 are fully (or almost fully) conserved and thus we cannot detect their coupling to other residues. In the GroEL–GroES complex, Leu27 in GroES is in direct contact with Val264 in the apical domain of GroEL.[7] The mutation Val264 → Ser in GroEL was found to block GroES binding.[27] Coupling of residues in GroES to Val264 in GroEL cannot be observed by us because this GroEL residue is absolutely conserved in our set of sequences. Interestingly, we find, however, that Leu27 in GroES is coupled to Met267 in GroEL (Fig. 2) which is not fully conserved and in direct contact with Val264. Ser35 in GroES which flanks the mobile loop is also found to be coupled to Met267.

Another pathway involves Ser21 in GroES which is in contact with Leu27 and is found to be coupled to Arg58 in GroEL which, in turn, is coupled to Met267. Mutations to Asp of the mobile loop residues Gly23 and Gly24 which are near Ser21 were found to lead to thermosensitive bacterial growth.[28] Mutations at position 24 were also found to affect GroES binding to GroEL[28,29] and to be suppressed by mutations in GroEL such as Val190 → Ile.[30] Val190 → Ile is also an intragenic suppressor mutation of Glu191 → Gly.[31] Other intragenic suppressor mutations of Glu191 → Gly include Arg322 → Gly and Thr331 → Ser, Ala, and Asn.[31] Our results show that Ser21 in GroES is coupled directly to Asp328 and indirectly via Arg58, Ala81, and Lys42 to Val323. Val323 and Asp328 are both close to the suppressor mutation sites Arg322 and Thr331 (Fig. 3). In other words, similar pathways are revealed by the genetic studies and correlated mutation analysis. Tyr71 is the fourth residue in GroES that is found to be coupled to residues in GroEL. It has been suggested[3] that this residue may be involved in polypeptide substrate binding in the GroEL–GroES *cis* complex which may account for its direct (to Asp328) and indirect (to Met267 and Ala356) coupling to apical domain residues in GroEL.

CONCLUSIONS

Analysis of correlated mutations in chaperonins has revealed networks of coupled residues that connect the equatorial domain with the intermediate and apical domains and with the co-chaperonin (GroES). Mapping of these networks onto the structures of GroEL[23] and the GroEL–GroES complex[7] and available genetic and biochemical data suggest that these networks are likely to reflect pathways of allosteric communication within and between GroEL subunits and between GroEL and GroES. These networks are consistent with information transfer from the equatorial to the apical domains and with the presence of intra-ring and inter-ring cooperativity. The partial overlap between the different networks is consistent with coupling between the different conformational changes that GroEL undergoes.[32,33] In principle, these networks may also reflect interactions between residues in the denatured state[24] or long-range electrostatic interactions.[25] However, the fact that many of the coupled residues are not charged and that the networks involve residues in both GroEL and GroES (which do not interact with each other in the denatured state) suggests that in many cases the coupling is attributable to conformational changes and that the networks observed here thus reflect pathways of allosteric communication. Two possible mechanisms (other than electrostatic interactions) of long-range propagation of conformational changes may be 1. changes in local fluctuations that affect global motion,[34] and 2. redistribution of conformational ensembles.[35] The main limitation of the approach in this study is that interactions between conserved (or nearly conserved) positions cannot be detected. A direct comparison of the results here with those of other studies is, thus, not always possible. Further support for our conclusions may in the future be provided by mutant cycle analysis[36] and theoretical work such as that based on the Gaussian network model.[34]

ACKNOWLEDGMENTS

A.H. is an incumbent of the Carl and Dorothy Bennett Professorial Chair in Biochemistry.

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