Compensatory Changes in GroEL/Gp31 Affinity as a Mechanism for Allele-specific Genetic Interaction*

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Previous work has shown that the GroEL-GroES interaction is primarily mediated by the GroES mobile loop. In bacteriophage T4 infection, GroES is substituted by the gene 31-encoded chaperonin, Gp31. Using a genetic selection scheme, we have identified a new set of mutations in gene 31 that affect interaction with GroEL; all mutations result in changes in the mobile loop of Gp31. Biochemical analyses reveal that the mobile loop mutations alter the affinity between Gp31 and GroEL, most likely by modulating the stability of the GroEL-bound hairpin conformation of the mobile loop. Surprisingly, mutations in groEL that display allele-specific interactions with mutations in gene 31 alter residues in the GroEL intermediate domain, distantly located from the mobile loop binding site. The observed patterns of genetic and biochemical interaction between GroES or Gp31 and GroEL point to a mechanism of genetic allele specificity based on compensatory changes in affinity of the protein-protein interaction. Mutations studied in this work indirectly alter affinity by modulating a folding transition in the Gp31 mobile loop or by modulating a hinged conformational change in GroEL.

Chaperonin-assisted folding of certain substrates depends on the coordinated interaction of GroEL, ATP, and GroES (1, 2). Certain unfolded or partially folded polypeptides bind to GroEL, a double-toroid, tetradecameric protein composed of 58-kDa subunits arranged in 7-fold symmetry (3–5). GroES, made up of 10.5-kDa subunits arranged in 7-fold symmetry (6, 7), binds to GroEL, thus stabilizing a conformational change that doubles the substrate-containing cavity of GroEL and promoting the release of the substrate into the cavity (8, 9). The amount of time the substrate spends in the cavity depends on the rate of ATP hydrolysis in the GroEL cis ring and the release of GroES (10, 11). In turn, the release of GroES is promoted by the binding of ATP or ATP and GroES to the trans GroEL ring (11, 12). After GroES release, the polypeptide substrate is released either in a folded or a folding-competent state or in a conformation still recognizable by GroEL, in which case it binds to the same or a different GroEL molecule (13). The efficient cycling of the GroE chaperone machine is essential to ensure that the chaperonin can provide the necessary folding assistance to its substrates (14–16).

GroEL is essential for bacteriophage T4 growth. The mutant groEL44(E191G) allele has been shown to block bacteriophage T4 growth at the level of capsid head assembly, i.e., in groEL44(E191G) mutant cells, Gp32, the major capsid protein, aggregates into amorphous lumps (17). The same phenotype was previously observed during infection of a wild type host by a bacteriophage T4 defective in gene 31 (18). Subsequent analyses of genetic suppressors identified an interaction between the host groEL gene and the bacteriophage T4 gene 31 (19).

It turned out that Gp31 is functionally analogous to GroES despite low sequence identity (14% at the amino acid level) (20, 21), and it can completely replace GroES for Escherichia coli growth. The crystal structures of GroES and Gp31 reveal significant structural identities as expected from their similar in vivo and in vitro function (6, 22). Both GroES and Gp31 subunits bear a flexible polypeptide segment, identified by nuclear magnetic resonance (NMR) spectroscopy and limited proteolysis (23, 24). The mobile loops mediate GroEL-GroES binding through a central hydrophobic tripeptide (Ile25–Val26–Leu27) as shown by NMR studies and confirmed by the crystal structure of the GroEL-ADP-GroES complex (8, 23).

All mutations identified thus far in either groES or gene 31, which result in defective GroEL interaction, alter amino acid residues in the mobile loop. Interestingly, most of these GroES substitutions do not affect the IVL tripeptide. Rather, the best characterized groES mutant alleles affect either of the two glycine residues preceding the IVL residues (25) and that participate in formation of a β-hairpin turn (24). Bacteriophage T4 gene 31 mutant alleles affect a number of residues in the mobile loop including residue Leu35, corresponding to Ile25 in the GroES mobile loop (26).

Mutations in groEL, originally identified on the basis of blocking bacteriophage growth, affect residues that are distant from the mobile-loop binding site. The affected residues lie in the intermediate domain that links the ATP binding equatorial domain with the substrate and GroES binding apical domain (23). The GroEL-ADP-GroES crystal structure revealed that the intermediate segment, in fact, provides two hinges that allow for the large en bloc movements in GroEL, which are captured by GroES binding (8). Genetic analyses have revealed that groEL mutations fall into two classes on the basis of their allele-specific interaction with groES (25, 27) or gene 31 (17) mutations. The study concerning GroEL-Gp31 interaction revealed that of two groEL mutants that block bacteriophage λ growth, groEL44(E191G) and groEL515(A383T), only the former blocks bacteriophage T4 growth. Surprisingly, mutations in gene 31

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31, that restore bacteriophage T4 growth on groEL44(E191G), simultaneously prevent plaque formation on groEL515(A383T).

Understanding how specific mutations in these genes affect chaperone function is the overall aim of the work described in this paper. Taking advantage of a simple genetic selection system, we have identified new mutations in gene 31 of bacteriophage T4 that switch the phenotype from suppression of groEL44(E191G) to suppression of groEL515(A383T). Biochemical analyses reveal that these gene 31 mutations exert their effects primarily by altering the affinity of GroEL for GroEL.

**EXPERIMENTAL PROCEDURES**

**Genetic Selection**—Twenty independent lysates of the original T4el(Gp31(L35I)) mutant were plated separately on groEL515(A383T) bacteria. Plaque formers, occurring at a frequency of approximately 10^-4 were isolated, restreaked, and characterized for plating ability on different groEL mutant hosts. The minimal gene 31 was amplified by polymerase chain reaction using Dynazyme Taq polymerase from a plaque isolated from a groEL515(A383T) lawn, and the polymerase chain reaction product was sequenced directly using the Amersham Pharmacia Biotech Delta Taq sequencing kit (28). All 20 suppressors sequenced contained the original El mutation (L35I) and in addition had a mutation that altered a second nearby amino acid residue, also localized in the mobile loop. Twelve of these pseudorevertants had a mutation that resulted in a change at codon 31 of Thr to Ala, and two candidates had a change at the same site from a Thr to an Ile.

**Cloning of Wild Type and Mutant Genes**—Mutant gene 31(L35I, T31A) was amplified by polymerase chain reaction; the polymerase chain reaction fragment was cloned into the EcoRI and XbaI sites of the high copy pBAD vector pPMMP201 (29), and the resulting clone overproducing Gp31(L35I,T31A) was named pALEX5. The wild type gene 31 was cloned in the same manner, except the amplification was done from a wild type T4 plaque isolated on a B178 lawn, and the resulting clone was named pALEX1. The Gp31(363W) protein was overproduced from pALEX2, created by the introduction of the corresponding mutation, resulting in 18.8 mM by site-directed mutagenesis (30) using pALEX1 as the template. Mutant gene 31 (L35I) was made by site-directed mutagenesis (30) using the plasmid pSV25 (wild type gene 31) as the template (20). All clones were sequenced in their entirety using either the standard Sanger sequencing method or automated sequencing (LiCORE).

pBADgroESgroEL is a plasmid that expresses the wild type groES and groEL genes under the control of the arabinose-induced promoter. The groES and groEL genes were cloned as a 2.1-kilobase pair fragment from pPMMP627 (27) containing 45 base pairs upstream of the starting ATG codon. The fragment was cloned into pBAD22 (31). Plasmid pBADgroESgroEL (A383T), used to overexpress GroEL (A383T), was constructed by replacing the BstXI-SmaI fragment from pBADgroESgroEL with the corresponding fragment from plasmid pOF1153 (27). The authenticity of the clone was verified by sequencing 300 base pairs around the altered codon.

**Protein Purification**—Wild type GroEL and GroES were overexpressed from pBADgroESgroEL transformed into MC1009 cells and induced with arabinose and purified essentially as described previously (25). Residual peptides bound to GroEL were removed by Affi-Gel Blue induced with arabinose and purified essentially as described previously.

**Citrate Synthase Refolding**—The chaperonin-dependent renaturation of pig heart citrate synthase (referred to in the text as citrate synthase) was performed as described previously (32). The following protein concentrations (given for monomers) were used: 4.2 μM chaperonin, 0.75 μM GroES, and 2 μM citrate synthase. Citrate synthase at 33 μM was denatured for 30–60 min at 27 °C in a solution containing 6 mM guanidine hydrochloride, 3 mM dithiothreitol, and 2 mM EDTA. The refolding buffer contained 10 mM MgCl₂, 2 mM ATP, 1 mM oxaloacetic acid, and 20 mM potassium phosphate, pH 7.4. The refolding reaction was run at 27 °C in a total volume of either 200 μl or 400 μl, and citrate synthase activity was measured after 60 min.

**Complex Formation**—Complex formation was initiated by adding ATP to a final concentration of 1 mM to a solution containing 50 mM Tris-HCl, pH 7.7, 7.8 mM MgCl₂, 1 mM KCl, 1 mM dithiothreitol, 8.4 μM GroEL, and 35.2 μM Gp31 (monomers). The reaction mixture (250 μl) was left at 22 °C for 10 min. An aliquot of 200 μl was loaded onto a TSK 3000G gel-filtration column that had been equilibrated in 50 mM Tris-HCl, pH 7.7, 10 mM MgCl₂, 1 mM KCl, 0.01% (w/v) Tween 20, and 0.25 mM ATP. The column was run at 22 °C at a flow rate of 1 ml/min. The fraction between 10.5 and 11.5 min was collected. Samples were acetone-precipitated and analyzed by means of electrophoresis on a 15% polyacrylamide gel containing SDS. The proteins were stained with Coomassie Brilliant Blue.

**Fluorescence Experiments**—A Photon Technologies Inc. QuantMaster luminescence spectrometer with a double excitation set-up and gloved cuvette holder maintained at 25 °C with a cooler-heater water bath system was used for fluorescence analysis. All reactions were performed under constant stirring and in a total volume of 2 ml. The intensity at 337 nm was monitored as a function of time with excitation at 295 nm and all slits adjusted to 4 nm. The following protein concentrations were used (in monomers): 2.0 μM GroEL, 1.4 μM Gp31(I36W), and 1.4 μM Gp31 and its mutant variants. ATP was used at 1 mM with a buffer containing 100 mM Tris-HCl, pH 7.5, 1 mM MgCl₂, and 1 mM dithiothreitol. Data were initially analyzed in Felix software provided with the spectrometer and converted into a Kaleidagraf for presentation. The background fluorescence intensity contribution associated with nontryptophan-containing proteins (expressed as percentage of the fluorescence intensity for Gp31(I36W) alone) was subtracted accordingly: GroEL, 20%; Gp31, 8%; Gp31(L35I), 5%; Gp31(L35I,T31A), 6%.

**Transfected Nuclear Overhauser Effect (trNOE)**—NMR Analysis—Carboxamide peptides corresponding to the mobile loops of Gp31, Gp31(L35I), and Gp31(L35I,T31A) were synthesized using 9-fluorenlyloxycarbonyl chemistry, acetylated off-line, and purified by reverse-phase high performance liquid chromatography. Sequences are as follows: Gp31, AQAGDVEEVSTEGILHKGRRQ; Gp31(L35I), AQAGDVEEVSTEGILHKGRRQ; Gp31(L35I,T31A), AQAGDVEEVSTEGILHKGRRQ. Peptide sequences were confirmed by matrix-assisted laser desorption ionization mass spectrometry (MALDI). GroEL and GroEL(E191G) were exchanged and concentrated into a 50 mM potassium phosphate, pH 6.1, buffer (Centricon-30, Amicon). GroEL was added to a final concentration of 60 μM in an NMR sample containing 2 mM peptide, 10% D₂O, and 0.3 mM trimethylsilylpropionate in 50 mM potassium phosphate, pH 6.1, buffer. Spectra were recorded at 30 °C on a General Electric Omega PG5 500 NMR spectrometer operating at 500.05 MHz frequency. Data were processed as described previously (24) using Felix software (Biouym Technologies, San Diego, CA) running on a Silicon Graphics Indigo (Mountain View, CA) work station.

**RESULTS**

GroEL/Gene 31 Allele-specific Mutations Fall into Two Classes—Previous work has shown that bacteriophage T4 mutations, which restore growth on groEL44(E191G) map to gene 31 (17), and sequencing of one candidate, called T4e1, showed that the mutation in gene 31 results in an amino acid change at codon 35 from Leu to Ile (26). This apparently subtle change in amino acid analysis or by the Bradford protein assay method, standard-ized with known concentrations of either GroEL or Gp31.

**The abbreviation used is: trNOE, transfected nuclear Overhauser effect.**
phage-encoded suppressors. From 20 independent T4 lysates plated on groEL515(A383T), bacteriophage “revertants” capable of forming a plaque were isolated at a frequency of approximately $10^{-6}$. All revertants simultaneously lost their capacity to propagate on groEL44(E191G) mutant bacteria. Sequence analysis showed that all 20 candidates retain the original $\varepsilon1$ mutation (Leu to Ile at codon 35). The most frequently occurring suppressor mutation (12 of 20 isolates) resulted in a substitution at codon 31 of Thr with Ala, and two additional revertants were shown to alter the same codon to an Ile (Table I). Significantly, the genetic interaction between residues 35 and 31 in the mobile loop of Gp31 coincides with the physical interaction observed in the GroEL-bound conformation determined by trNOE NMR (Fig. 5A) (24).

**Table I**

| groEL wild type | groEL44 (E191G) | GroEL515 (A383T) |
|-----------------|-----------------|------------------|
| T4 wild type    | +               | +                |
| T4J(L35I)       | +               | +                |
| T4J(L35I,T31A)  | +               | +                |
| 12 of 20 isolates | -             | -                |
| T4J(L35I,T31I)  | +               | +                |
| 2 of 20 isolates | -               | -                |

Mutations in groEL and Gene 31 Affect Chaperonin-assisted Refolding of Citrate Synthase—Previous work has shown that citrate synthase depends on both GroEL and GroES for renaturation (32). In the absence of chaperonins, only 10–20% of denatured citrate synthase regains activity. GroEL alone (with ATP) inhibits refolding of citrate synthase (Fig. 1). In contrast, GroEL paired with GroES, Gp31, Gp31(L35I), or Gp31(L35I, T31A) efficiently helps citrate synthase refolding. Likewise, GroEL(E191G) inhibits refolding of the substrate in the absence of a chaperonin but assists citrate synthase refolding with GroES. However, when paired with Gp31, GroEL(E191G) is unable to assist refolding. As anticipated from our in vivo genetic analysis, Gp31(L35I) restores chaperonin-assisted folding by GroEL(E191G), whereas Gp31(L35I,T31A) and GroEL(E191G) form a nonfunctional pair for the refolding of citrate synthase.

The above results indicate that citrate synthase refolding depends on functional interaction between GroEL and Gp31, strongly suggesting that the observed defects in bacteriophage T4 growth are also the result of aberrant chaperone-assisted folding. What is the molecular basis of the defect in chaperonin-cochaperonin interaction? The amino acid substitutions in Gp31 or GroEL could increase or decrease their affinity for each other. To test this, we analyzed complex formation by gel filtration chromatography.

**The L35I Substitution in Gp31 Restores Binding to GroEL(E191G)—**Formation of the GroEL-Gp31 complex requires nucleotide and can be observed by size fractionation on gel filtration chromatography (Fig. 2A). With the same conditions, GroEL(E191G) does not form a complex with Gp31 (Fig. 2B). Therefore, the inability of GroEL(E191G) to assist citrate synthase refolding when paired with Gp31 is most likely because of a lack of stable chaperonin-cochaperonin complex formation. However, GroEL(E191G)+ATP does form a stable complex with Gp31(L35I) (Fig. 2C). Thus, we conclude that the substitution in Gp31(L35I) increases the affinity of Gp31 affinity for GroEL, and we hypothesize that Gp31(L35I,T31A) decreases the affinity of Gp31 for GroEL. To test this, we developed a technique to distinguish subtle differences in GroEL binding between Gp31 and Gp31 mutants.

Relative GroEL-binding Affinities of Gp31 Mutants Determined by Competition with a Fluorescent Gp31 Variant—Tryptophan fluorescence may be exploited to measure protein-protein interactions provided that the tryptophan undergoes an environmental change upon formation of the complex. Because neither GroEL, GroES, nor Gp31 contain tryptophan residues, we sought to introduce one such that it would report Gp31 binding to GroEL. Gp31(I36W) was created by site-directed mutagenesis. Ile$^{36}$ is the central residue of the hydrophobic tripeptide, Leu$^{35}$-Ile$^{36}$-Ile$^{37}$, in the Gp31 mobile loop. Gp31(I36W) interacts with GroEL in a manner similar to wild type Gp31, consistent with the fact that it can substitute for wild type Gp31 in bacteriophage T4 growth. However, unlike
wild type Gp31, Gp31(I36W) functions with GroEL(E191G) in citrate synthase refolding (Fig. 1). Nevertheless, Gp31(I36W) can be exploited as a reporter to detect the relative binding affinities of the various GroES and Gp31 proteins in a competition assay.

The fluorescence emission spectrum of Gp31(I36W) exhibits a wavelength of maximum emission ($\lambda_{\text{max}}$) of 347 nm, which suggests that the introduced tryptophan side chain is solvent-exposed (Fig. 3A). The addition of GroEL in the absence of nucleotide results in a small increase in fluorescence intensity and no change in emission $\lambda_{\text{max}}$. However, further addition of ATP results in a 2-fold increase in emission intensity as well as a 10-nm decrease in $\lambda_{\text{max}}$. These results suggest that the tryptophan side chain is transferred to a nonpolar environment in the GroEL-Gp31 complex.

The relative binding affinities of Gp31 and Gp31 mutants can be evaluated with a binding competition assay. First, a complex between GroEL and Gp31 is formed in the presence of nucleotide. Subsequently, binding of Gp31(I36W) to GroEL is monitored by tryptophan fluorescence. The extent that Gp31 blocks the change in fluorescence indicates the ability of the Gp31 competitor to inhibit Gp31(I36W) binding.

Prior incubation of GroEL-ATP with Gp31 only slightly inhibits Gp31(I36W) binding (Fig. 3B). In contrast, Gp31(L35I) complexed with GroEL-ATP effectively blocks Gp31(I36W) binding, indicating that it is more difficult to displace than its wild type counterpart. Gp31(L35I,T31A) in complex with GroEL-ATP hinders Gp31(I36W) binding partially, neither as well as Gp31(L35I) nor as weakly as Gp31. From these experiments, the following relative order of cochaperonin binding affinity to GroEL-ATP is established: Gp31(L35I) > Gp31(L35I,T31A) > Gp31.

Because the affected residues in the GroEL mutants are distant from the mobile loop binding site, one would expect the same order of binding affinity on the GroEL mutants used in this study. We tested this prediction by repeating the same experiment while substituting GroEL with GroEL(A383T). Indeed, the order of relative affinity remains the same (data not shown).

**Synthetic Mobile Loop Peptides Corresponding to Gp31 Mutants Recapitulate Altered GroEL Binding**—Above we showed that mutations that affect residues in the mobile loop of Gp31 alter its affinity for GroEL. To establish that these differences are a direct result of changes in the mobile loop binding to GroEL, we compared the GroEL binding properties of synthetic peptides corresponding to the mobile loops of our Gp31 mutants by analysis of trNOEs. The appearance of trNOEs in the NOESY spectra of the three peptides in the presence of GroEL indicates that each binds to GroEL (Fig. 4). However, the Gp31(L35I) peptide exhibits more intense trNOEs compared with those of Gp31 and Gp31(L35I,T31A) peptides, suggesting that the L35I substitution strengthens mobile loop binding to GroEL and that the T31A substitution weakens it. In the presence of GroEL(E191G), there is a marked decrease in trNOEs for Gp31 and Gp31(L35I,T31A) compared with Gp31(L35I), consistent with the lack of *in vivo* interaction between GroEL(E191G) and these cochaperonins. These results indicate that the mobile loop itself can recognize the defect in GroEL(E191G) despite the relatively large distance between the altered amino acid residue and the mobile loop binding site in GroEL (Fig. 5A).

**DISCUSSION**

**GroEL-Gp31 Mutant-Suppressor Pairs Illustrate a Mechanism of Allele-specific Genetic Interaction**—As stated earlier, taking advantage of a GroEL mutant, GroEL(A383T), that does not function with Gp31(L35I), we have isolated compensatory mutations that reveal a striking genetic interaction between Gp31 and GroEL. Specifically, all mutants of bacteriophage T4 Gp31(L35I) isolated as restoring ability to grow on...
groEL515(A383T) simultaneously lose their ability to grow on

groEL44(E191G). Potentially, the GroEL-Gp31 genetic in-
novation could be ascribed to a conventional mechanism of allele

specificity, in which distinct mutant-suppressor pairs arise

from direct contacts among the affected amino acids in the

protein-protein interface (33). However, amino acids involved

in the GroEL-Gp31 genetic interactions analyzed here are lo-

cated far from each other in the GroEL-Gp31 complex (Fig. 5

A).

Our biochemical analyses of the mutant Gp31 proteins sug-

gest that mutant-suppressor pairs complement each other by

contributing in opposite ways to GroEL-Gp31 affinity. The

relative affinities of wild type and mutant Gp31 proteins were

probed indirectly through their ability to block binding to

GroEL of a fluorescent variant of Gp31, Gp31(I36W), and by

the strength of trNOEs observed for their corresponding mobile

loop peptides in the presence of GroEL. Both assays indicate

the following relative affinity for GroEL: Gp31(L35I) >

Gp31(L35I,T31A) > Gp31. Thus, it appears that the increased

GroEL binding affinity of Gp31(L35I) compensates for the low

affinity interaction of the GroEL(E191G)-Gp31 pair, and the

reduced affinity of Gp31(L35I,T31A) compensates for the pre-

sumed high affinity interaction of the GroEL(A383T)-

Gp31(L35I) pair. The same relative affinity ranking is observed

for binding of the various Gp31 proteins to GroEL(A383T) (data

not shown) and for binding of the corresponding mobile loop

peptides to GroEL(E191G). The fact that the order of affinity is

indifferent to the GroEL protein tested supports our proposal

that the mechanism of GroEL-Gp31 genetic interactions stud-

ied here arises from compensatory affinity changes rather than

classical allele-specific alterations in the structure of the bind-

ing interface.

Amino Acid Substitutions in the Mobile Loop Affect Forma-

tion of the GroEL-bound Hairpin Conformation—As detailed

above, the predominance of substitutions at amino acid posi-

tion 31 in the pseudorevertants (14 of 20 isolates; Table I)

suggests that changes at this position have a greater potential

for counteracting the increased binding affinity caused by the

L35I substitution. Consistent with this hypothesis, previous

NMR studies detected trNOEs between the side chains of Thr31

FIG. 5. X-ray and NMR structures. A, a single subunit from the

GroEL-ADPGroES crystal structure (8). The GroEL/E191G) and

GroEL(A383T) specific amino acid changes are localized in the hinges

flanking the intermediate domain of GroEL and are highlighted. B, the

structure of the GroEL-bound GroES mobile loop peptide was deter-

mined by trNOE NMR in the presence of GroEL. The Ser21 and Ile25

residues (equivalent to Thr31 and Leu35 of Gp31, respectively) interact

in the GroEL-bound conformation (24). Structures were modeled using

MolScript (44).
and Leu\textsuperscript{35} in the Gp31 mobile loop peptide bound to GroEL, indicating that these side chains approach within a few angstroms of each other (24). The proposed Thr\textsuperscript{31}-Leu\textsuperscript{35} contact corresponds to the Ser\textsuperscript{21}-Ile\textsuperscript{25} contact observed in the GroEL-bound GroES mobile loop peptide (23). All of the trNOE data in the Gp31 peptide were consistent with its forming the same 3:5 hairpin conformation (using the classification of Sibanda and Thornton (34)) as that formed by the corresponding GroES mobile loop peptide (Fig. 5B), which places these two side chains next to each other but on opposite strands of the hairpin.

Amino acid \(\beta\)-strand preferences can account for the observed changes in GroEL-Gp31 binding caused by the various mutations. A host-guest study ranked all 20 amino acids by the change in free energy for folding of a protein in which the substituted site was located at the edge of a \(\beta\)-sheet (35). This study may be the most relevant because a \(\beta\)-hairpin more closely resembles the edge than the middle of a \(\beta\)-sheet. It was found that Leu is unfavorable for \(\beta\)-sheet formation, Ala and Ile bridge, whereas Thr is favorable. Thus, the L35I substitution is expected to increase \(\beta\)-hairpin stability, whereas either T31A or T31I are expected to decrease \(\beta\)-hairpin stability. Because Gp31 binding to GroEL is coupled to \(\beta\)-hairpin formation, changes in GroEL binding affinity may result from changes in \(\beta\)-hairpin stability. Minor and Kim (35) noted a significant difference in the rank order of amino acids in \(\beta\)-sheet propensity for substitution at the edge of a \(\beta\)-sheet versus the middle of a \(\beta\)-sheet. In particular, Ile is a strong \(\beta\)-sheet-former in the middle of a \(\beta\)-sheet but essentially neutral at its edge. The authors suggested this is because of a balance of a favorable contribution from side chain conformational entropy and poor hydrophobic burial at the edge of the \(\beta\)-sheet. Thus, the initially surprising result that either the T31A or T31I substitution can result in the same phenotype can now be understood in terms of contributions to \(\beta\)-sheet stability.

Our experimental results, coupled with the conclusions derived from the model systems discussed above, demonstrate that the GroEL-Gp31 genetic interaction can be understood in the framework of a folding transition by the Gp31 mobile loop. The mobile loop conformational dynamics must be exquisitely poised for folding into a \(\beta\)-hairpin and yet sufficiently disordered that GroEL binding is not too tight. As a result, seemingly subtle amino acid substitutions, such as L35I, can completely rescue or block bacteriophage T4 growth depending on the particular mutant host.

Amino Acid Substitutions in the GroEL Hinge Regions Affect Local as Well as Large Scale Conformational Changes—Substitutions in the hinges could change the distribution of GroEL subunits between apical domain-open and apical domain-closed conformations and/or affect the apical domain-mobile loop docking interaction by allosteric communication. We compared the relative orientation of the three GroEL apical domain residues that contact the mobile loop in the crystal structure of GroEL-ADP-GroES with the orientation of these residues in the crystal structure of GroEL alone (Fig. 6 (8, 36)). The orientation of the Val\textsuperscript{264} side chain is shifted with respect to other apical domain residues, suggesting that the \(\alpha\)-helix containing Val\textsuperscript{264} twists when GroEL visits its open conformation. The other two GroEL residues, Leu\textsuperscript{234} and Leu\textsuperscript{237}, show minor changes in orientation. Because mobile loop peptides exhibit reduced binding to GroEL(E191G), mobile loop and, therefore, GroES binding may be controlled by the ratio of open versus closed GroEL subunits. If the mobile loop has a lower affinity for GroEL in the closed conformation and GroEL(E191G) visits the open state less frequently, then poor binding of Gp31 could be because of a smaller population of GroEL(E191G) subunits in the open state.

Implications for Protein-Protein Interaction—An affinity-based mechanism for allele specificity has been ascribed to other protein-protein interactions, raising the possibility that the classical notion of allele-specificity is generally avoided by robust, flexible protein-protein contacts. For example, suppression of defects in fimbrin-actin interactions in yeast has been attributed to a global increase in affinity of fimbrin mutants for actin mutants, and at least two fimbrin mutants bind more tightly to wild type actin (37). Crystal structures of human fimbrin and bovine actin reveal that residues affected in the mutant yeast proteins are not only localized to surfaces of potential protein-protein contact, and several are buried in a hydrophobic core (38). In the bacterial chemotaxis system, interaction of the response regulator CheY with the receptor kinase CheA has been localized to a surface of CheY, but residues in the contact surface are not evolutionarily conserved, and crystal structures of CheY with the P2 domain of CheA reveal at least three different modes of binding (39, 40). In the chaperonin system, we find that mutations affecting GroEL-Gp31 affinity modulate a folding transition in the Gp31 mobile loop. Hence, in all of these systems, residues in the intermolecular interfaces may be less critical than residues controlling domain folding and stability.

Strict allele-specificity may always involve a conformational switch in one partner of a protein-protein interacting pair. In the chemotaxis system, bias for clockwise versus counter-clockwise motor rotation is controlled by interactions of phosphorylated CheY with the flagellar switch protein FlIM. Many of not all mutations in FlIM that suppress mutations in CheY are thought to adjust the bias of the switch rather than restore normal interactions with mutant CheY (41, 42). The indirect effect of these suppressor mutations in FlIM is analogous to the effect of hinge mutations in GroEL, that compensate for strong or weak binding by mobile loop mutants in Gp31. Apparently, a great deal of redundancy has accumulated in the structural
features of protein-protein interactions, as has already been appreciated in protein folding itself (43). Mutations tend to shift conformational equilibria between broad energy minima rather than cause distinct changes in structure.

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