An ORFan no more: The bacteriophage T4 39.2 gene product, NwgI, modulates GroEL chaperone function*

Debbie Ang and Costa Georgopoulos

Department of Biochemistry, University of Utah, Salt Lake City UT 84112-5650

*This article is dedicated to the memory of our dear friend and colleague Richard (Dick) Epstein (March 14, 1929-March 6, 2011), the discoverer of the bacteriophage T4 amber mutations and an inspiration to generations of geneticists.
Running title: The T4 39.2 (NwgI) protein modulates GroEL

Key words: GroEL (Hsp60)/GroES (Hsp10)/Gp31/T4-like bacteriophages/39.2 (NwgI)

Corresponding author:
Costa Georgopoulos
Dept. of Biochemistry, University of Utah, 15 N Medical Drive East, RM 4100,
Salt Lake City UT 84112-5650

tel: 801-581-8033
fax: 801-581-7959
costa@biochem.utah.edu
Abstract

Bacteriophages are the most abundant biological entities in our biosphere, characterized by their hyperplasticity, mosaic composition, and the many unknown functions (ORFans) encoded by their immense genetic repertoire. These genes are potentially maintained by the bacteriophage to allow efficient propagation on hosts encountered in nature. To test this hypothesis, we devised a selection to identify bacteriophage-encoded gene(s) that modulate the host Escherichia coli GroEL/GroES chaperone machine, which is essential for the folding of certain host and bacteriophage proteins. As a result, we identified the bacteriophage RB69 gene 39.2, of previously unknown function, and showed that homologs of 39.2 in bacteriophages T4, RB43 and RB49 similarly modulate GroEL/GroES. Production of wild type bacteriophage T4 Gp39.2, a 58 amino acid protein, (a) enables diverse bacteriophages to plaque on the otherwise nonpermissive groES or groEL mutant hosts in an allele-specific manner, (b) suppresses the temperature-sensitive phenotype of both groES and groEL mutants, (c) suppresses the defective UV-induced PolV function (UmuCD) of the groELA44 mutant, and (d) is lethal to the host when overproduced. Finally, as proof of principle that Gp39.2 is essential for bacteriophage growth on certain bacterial hosts, we constructed a T4 39.2 deletion strain and showed that, unlike the isogenic wild type parent, it is incapable of propagating on certain groEL mutant hosts. We propose a model of how Gp39.2 modulates GroES/GroEL function.
Introduction

During the 1940's, Alfred Hershey's pioneering experiments led to the isolation and characterization of bacteriophage T2 rapid lysis mutants (Hershey 1946; Hershey and Rotman 1948). Seymour Benzer exploited the corresponding bacteriophage T4 rII class of mutants (Lederberg and Lederberg 1953) in his classic experiments on genetic fine structure through DNA recombination, thus redefining our concept of the gene, the cistron, and the various distinct classes of mutations (Benzer 1955, 1959). His work clearly showed that T4 possesses two genes, rIIA and rIIB, which are essential for growth on bacteriophage λ lysogens, but not on non-lysogens.

Shortly thereafter, Dick Epstein, based on his Ph.D. thesis work with UV-inactivation curves of T4 on E. coli B and K-12 (λ), hypothesized that, in an analogous manner, T4 may possess gene(s) essential for growth on E. coli B but not K-12 (λ) (Epstein 1958a, 1958b). The experiment testing this hypothesis led to the classic discovery of the amber (nonsense) mutants (Epstein et al. 1964, this issue; Stahl 1995), also independently discovered by Allan Campbell with bacteriophage λ (Campbell 1961). Although Dick Epstein's original idea that bacteriophage T4 possesses genes that specifically enable it to grow on E. coli B but not E. coli K-12 (λ) is thus far unsubstantiated, recent genetic analyses coupled with rapid DNA sequencing techniques have indeed shown that even closely related bacteriophages carry their own sets of unique genes that most likely favor their growth on certain bacterial hosts in nature (see below).

Recently there has been renewed interest in the vast number and variety of bacteriophages found in the environment (reviewed in Wommack and Colwell 2000; Hendrix 2002; Rohwer and
Edwards 2002; Casjens 2008). Studies of related bacteriophages show that while genes encoding morphological components such as capsid proteins and tail fibers tend to be conserved, the rest of the genome displays remarkable diversity. One of the most interesting findings is the existence of novel open reading frames (referred to as ORFans; Fischer and Eisenberg 1999) whose functions are unknown and that have no significant homology to other sequences currently found in databases (Nolan et al. 2006; Comeau et al. 2007, 2008, 2010; Zuber et al. 2007; Hatfull 2008; Denou et al. 2009; Petrov et al. 2010). Whereas many of these ORFan genes are nonessential under standard laboratory conditions, their occasional presence in related bacteriophages suggests that they are important for survival in the wild. The difficulty lies in determining the functions of these seemingly nonessential genes, whose effects may be subtle or observable only under specific conditions.

Because each bacteriophage relies heavily on its bacterial host(s) for propagation, studying this relationship has offered many insights into fundamental bacteriophage and host functions. For example, the GroEL/GroES chaperone machine of *E. coli* was originally discovered through host mutations that block the propagation of certain bacteriophages (reviewed in Friedman et al. 1984; Ang et al. 2000; Georgopoulos 2006). Subsequent work showed that the groEL and groES genes form an operon and are coordinately expressed under normal housekeeping conditions. Their expression is increased under conditions of stress such as heat shock (reviewed in Zeilstra-Ryalls et al. 1991; Georgopoulos 2006). The importance of the GroES/GroEL chaperone machine is exemplified by the fact that it is almost universally conserved and highly homologous to the essential Hsp10/Hsp60 proteins residing in chloroplasts and our own mitochondria (Horwich and Fenton 2009; Hartl et al. 2011). Furthermore, it is the only chaperone machine assisting the
correct folding of certain essential proteins in *E. coli* (Houry et al. 1999; Kerner et al. 2005), thus is itself absolutely essential for life (Fayet et al. 1989).

Electron microscopy and x-ray crystallography showed that GroEL's native structure is a tetradecamer composed of two back-to-back heptameric rings (reviewed in Sigler et al. 1998). The much smaller GroES cochaperone is a heptamer that binds to one or both ends of GroEL through its mobile loops (Landry et al. 1993; Sigler et al. 1998). GroEL captures unfolded substrates via the hydrophobic surface of the apical domains of one of its rings (referred to as the *cis* ring). Subsequent ATP binding at the *cis* ring of GroEL leads to massive *en bloc* rearrangements of the GroEL subunits, leading to GroES binding and release of the substrate into the GroEL-GroES cavity, whose interior is now lined with mostly hydrophilic residues (Xu et al. 1997; Sigler et al. 1998). Some of the apical domain hydrophobic groups now exposed on the GroEL surface are bound by the seven mobile loops of the GroES cochaperone. This GroEL/cochaperone interaction is the result of a delicate balance between disorder and order of the cochaperone mobile loops (Landry et al. 1993, 1996; Richardson and Georopoulos 1999; Richardson et al. 1999; Shewmaker et al. 2001, 2004). When bound to GroEL, the disordered cochaperone mobile loops become structurally ordered, although physical interaction with GroEL occurs only through a universally conserved tripeptide in the mobile loop (Landry et al. 1993, 1996; Xu et al. 1997). Earlier work showed that all of our groES mutations alter residues in the mobile loop, resulting in either weakened or strengthened interactions with GroEL (Landry et al. 1993, 1996; Zeilstra-Ryalls et al. 1996; Richardson et al. 1999; Richardson 2000; Shewmaker et al. 2004). The as yet unfolded released substrate in the cavity has approximately 10 sec, the average time required for ATP hydrolysis, to reach a folding-competent conformation. ATP
hydrolysis results in binding of ATP/substrate to the \textit{trans} ring, thus releasing GroES and the folded substrate from the \textit{cis} ring (Sigler \textit{et al.} 1998; Horwich and Fenton 2009; Hartl \textit{et al.} 2011).

Interestingly, whereas the temperate bacteriophage \(\lambda\) requires both the \textit{groES} and \textit{groEL} gene products for the correct assembly of its prohead, bacteriophage T4 does not require GroES because it encodes its own cochaperone called Gp31, which is distantly related to GroES (<10\% sequence identity) (Nivinskas and Black 1988; Keppel \textit{et al.} 1990; Koonin and Van Der Vies 1995). Many of the so-called T4-like bacteriophages encode Gp31 homologs, some of which are capable of substituting for the \textit{E. coli} GroES protein in host function (Ang \textit{et al.} 2000; Ang \textit{et al.} 2001; Keppel \textit{et al.} 2002). Despite the lack of sequence similarity, both GroES and Gp31 assemble into very similar structures (Hunt \textit{et al.} 1996; Hunt \textit{et al.} 1997). However, the cavity found in the GroEL/Gp31 complex is larger than that formed with GroES (Hunt \textit{et al.} 1996; Landry \textit{et al.} 1996; Hunt \textit{et al.} 1997; Clare \textit{et al.} 2009). The observed differences may have evolved to accommodate the major bacteriophage coat protein Gp23, which, at approximately 56,000 Da, is at the upper limit for the cavity of the GroEL/GroES complex. As opposed to other GroEL-bound substrates, Gp23 seems to occupy a unique position under the GroEL/Gp31 dome (Clare \textit{et al.} 2009). Furthermore, Snyder and Tarkowski (2005) have presented evidence suggesting that the nascent N-terminal region of Gp23 targets it directly to GroEL, thus potentially speeding up the rate of folding during bacteriophage T4 intracellular growth.

To better understand the relationship between T4 and its host \textit{E. coli}, we have used a direct genetic selection to identify additional bacteriophage genes that regulate GroE function. By using various \textit{E. coli groEL} and \textit{groES} mutants to screen a bacteriophage \(\lambda\) DNA library of the T4-like
bacteriophage RB69, we identified the product of gene 39.2 as a specific modulator of GroE function. By testing its effect on a variety of host and bacteriophage mutants \textit{in vivo} we conclude that the ability of the Gp39.2 protein to suppress GroE defects is limited to situations where either the GroEL or its cochaperone mutant exhibits a weakened affinity for the other partner. Finally, we show that deletion of the 39.2 gene from the T4 genome inhibits its growth on certain \textit{E. coli} hosts, potentially justifying its retention in the genome.
**Materials and Methods**

*Strains and plasmids*

See Table 1 for a list of strains, bacteriophages and plasmids used in this study. Bacteriophages RB69, RB49, and RB43 were kind gifts of Dr. Henry Krisch (Toulouse). Bacteriophage T4 K10 and plasmid pBSPLO (-) were kind gifts of Dr. Ken Kreuzer (Duke University). Cells were grown in L-broth (10 g tryptone, 5 g yeast extract, 5 g NaCl per liter, pH 7) or on L-plates (L-broth with 10 g agar/liter) with antibiotics where appropriate (final concentrations: 100 µg/ml ampicillin; 50 µg/ml kanamycin; 10 µg/ml chloramphenicol).

*Isolation of λZAP II clones with the ability to propagate on groE mutants*

A λZAP II library of RB69 DNA (prepared by Stratagene; a gift from Dr. Jim Karam, Tulane University) was used to infect fresh overnight cultures of *E. coli* B178 groEL44, groEL515, and groES42 at a multiplicity of infection (m.o.i.) of 0.01. Following incubation at room temperature for adsorption, the infected cells were mixed with 3 ml molten soft agar (L-broth with 0.6% agar), poured onto fresh L-plates and incubated at 37° overnight. The next day, plaques were purified according to standard procedures, followed by preparation of stock lysates. Growth on the corresponding mutant host was confirmed prior to further characterization.

*Identification of the RB69 39.2 open reading frame*

To identify the region(s) of the RB69 genome represented by the λZAP II clones, DNA was isolated from each bacteriophage candidate by freeze-thaw, i.e., 95° for 10 min, -20° for 20 min, 95° for 10 min, and then used as a template in a standard PCR reaction according to the
manufacturer's protocol (Pfx; GibcoBRL/Invitrogen; primers forward: 5’ ccc agt cac gac gtt gta aaa cg and reverse: 5’ agc gga taa caa ttt cac aca gg). Gel-purified PCR products (Qiagen) were sequenced from the ends using forward and reverse primers, followed by BLASTn comparison (Altschul and Lipman 1990) to identify the genomic region represented by each clone. Subcloning of individual open reading frames into the λZAP II vector allowed us to identify the gene of interest.

Cloning and mutagenesis of T4 39.2

The 430 bp region encompassing the T4 39.2 open reading frame was initially PCR-amplified from genomic DNA using primers derived from the published sequences of Huang (1986) and Sanson and Uzan (1992): 5’gca gga aga gaa aga gca aga aca aat ag and 5’gaa cca att ata tct aga gat ttc aat att ag. Following sequence verification, the minimal gene was then PCR-amplified from genomic DNA and cloned into vector pMPMK4 (Mayer 1995) using restriction sites EcoRI and PstI, thus placing the gene under the regulation of the P_{BAD} arabinose-inducible promoter (Guzman et al. 1995; Mayer 1995). The primers used to amplify the minimal T4 39.2 gene were: 5’ gtt gag gaa ttc acc atg ccg ctt tat gat and 5’ ggt ggt ctg cag tca tta ccc ttt aag caa gtc gta. The resulting plasmid was named pAC.

Site-directed mutagenesis of the C13 and the C31, C34 residue codons in T4 39.2 was accomplished using the Quik-Change method (Stratagene) and pAC as template. Primers 5’ caa tcc aaa gac gct gca aaa gaa tac g and 5’ cgt att ctt ttg cag cgt ctt tgg att g were used for the C13A mutation. Primers 5’ ctg aaa gag ata ctg atg tag ctc ctg atg ctc atc ggc tgg ctg ttc and 5’ gaa cag
CCA GCC GAT GAG CAT CAG GAG CTA CAT CAG TAT CTC TTT CAG were used for the C31A C34A mutations. The resulting plasmids were named pDA1844 and pDA1846, respectively.

**Homologs of 39.2 in other T4-like bacteriophages**

To test if other T4-like bacteriophages encode 39.2, DNA from T4, RB49, RB43, T2, T6, OX2 and K3 was PCR-amplified using primers for the minimal T4 39.2 gene: 5’gct tac ttt aag gaa tac ata tgc cgc ttt atg at and 5’ggt ggt gga tcc tta ccc ttt aag caa gta gta. Products were processed and sequenced as before. The RB49 and RB43 39.2 homologs were PCR-amplified using primer pairs 5’ gtt gag gaa ttc acc atg cca tta tac gat tat and 5’ ggt ggt ctc gac gac tca tga cac gfc cac, and 5’ gct tac tct cac gaa tac ata tga ccc tta caa cat ata cc and 5’gat cgt aga cgt tct aga tat ttt aga cat agg g, respectively. The RB49 39.2 gene was cloned into pMPMK4 at the EcoRI and PstI sites, yielding plasmid pAQ.

**Construction of a T4 39.2 deletion mutant**

A 39.2 null mutation in bacteriophage T4 was constructed using the method of Selick et al. (1988). Essentially, the regions flanking 39.2 in T4 were amplified in two separate reactions according to the manufacturer’s protocol (Pfx; Invitrogen), using primer pairs **no. 1** - 5’ctg ata acg gat cct atg cac and **no. 2** - 5’tat att tca taa tta cga gct ccc ggg cat atg aat tat tcc tta aag, and **no. 3** - 5’ctt taa gga ata att cat atg ccc ggg agc tgc taa tta tga aat ata and **no. 4** - 5’gtt tcg cta gca gaa gag cca at. The two gel-purified products, which share homology in the region spanning the deletion of 39.2, were fused in a single standard PCR reaction (Pfx; Invitrogen) using primers no. 1 and 4. The product was digested with BamHI and NheI, and cloned into pBSPLO (-) digested with BamHI and XbaI, resulting in plasmid pAA. Strain C600 (supE44), transformed with pAA, was infected
with bacteriophage T4 K10 which carries amber mutations in two essential genes, 38 and 51 (Selick et al. 1988). The resulting lysate was plated on lawns of E. coli B178 (sup°) to select integrants, i.e., bacteriophage that have integrated the plasmid by homologous recombination at the site of 39.2. [pBSPLO (-) encodes supF which suppresses the amber mutations in T4 K10 in a sup° host.] Candidates were purified on B178 lawns to isolate single plaques. To recover plasmid-free bacteriophage, single plaques were resuspended in L-broth and used to infect C600 bacteria. The resulting lysates were plated on C600 lawns. Plaques were screened on B178 and C600 lawns to find those that had lost the plasmid (growth only on C600). Candidates were further screened by PCR for loss of the 39.2 allele. Four out of 17 sequenced candidates had lost the 39.2 gene. One of these T4 K10 Δ39.2 bacteriophages was extensively backcrossed to wild type T4Do, continuously selecting for loss of the amber mutations and screening by PCR for the simultaneous loss of the 39.2 gene to arrive at the isogenic T4 Δ39.2 bacteriophage used in this work.

Selection of rifamycin-resistant mutants

To determine the frequency of rifamycin-resistant mutants in UV-irradiated cultures, we essentially followed the protocol of Donnelly and Walker (1989). Overnight cultures were diluted 1:100 into LB and incubated at 37°C with aeration. At OD_{595nm} 0.2, arabinose was added to the appropriate cultures to a final concentration of 0.02%. Incubation at 37°C was continued for all cultures until they reached approximately OD_{595nm} 0.8. Cells were centrifuged and resuspended in an equal volume of 10 mM MgSO₄. For each time point, a 1-ml aliquot of cells was placed on the sterile surface of a plastic Petri dish lid and covered with aluminum foil. The lid was positioned under a prewarmed UV lamp, the foil removed and counting of time begun. Cells were transferred
to 1.5-ml microfuge tubes on ice and kept covered with foil whenever possible to prevent photoreactivation. Dilutions were spread on L-plates to determine rates of survival. In addition, 0.25 ml of each time point was diluted into 5 ml L-broth to allow outgrowth of rifamycin-resistant mutants. The tubes were covered with aluminum foil and incubated at 37° overnight with aeration. Dilutions were spread on L-plates with or without rifamycin (100 µg/ml) and incubated overnight at 37°C. Colonies were counted the next day to determine the frequency of rifamycin-resistant mutants.
Results

Selection of RB69 clones that allow bacteriophage growth on E. coli groE mutant strains

*E. coli* groES and groEL mutants were first identified by their resistance to either bacteriophage λ. or T4 infection (reviewed in Friedman et al. 1984; Zeilstra-Ryalls et al. 1991; Ang et al. 2000; Georgopoulos 2006). Later work showed that, whereas both GroEL and GroES are required for λ head morphogenesis, only GroEL is required for the folding of bacteriophage T4's major capsid protein Gp23. GroES is not required because T4 encodes its own distant cochaperone ortholog named Gp31 (Nivinskas and Black 1988; Keppel et al. 1990; Van Der Vies et al. 1994). Our interest in homologs of T4 Gp31 led us to the λZAP II library carrying 4-10 kbp fragments of genomic DNA isolated from the T4-like bacteriophage RB69, which we used to infect lawns of *E. coli* groEL44, groEL515 and groES42 mutants. From our previous studies, we knew that T4 Gp31 expressed from a plasmid allows bacteriophage λ to propagate on our groES mutants, as well as the groEL515 mutant (Keppel et al. 2002). Thus, we expected to identify the RB69 homolog of the T4 31 cochaperone gene by infecting the groES42 and groEL515 mutants with the library and isolating plaque-formers.

We had shown earlier that bacteriophage T4 growth is blocked on the groEL44 mutant because its Gp31 product does not interact with the mutant GroEL44 protein (Shewmaker et al. 2004). As such, we used *E. coli* groEL44 as a negative control here, under the assumption that the putative RB69 Gp31 homolog likewise would not interact with GroEL44.
Consistent with our prediction, on *E. coli* groES42 lawns we isolated λZAP II plaque-formers from the RB69 DNA library at the expected frequency of $3 \times 10^{-3}$ per plaque-former on wild type *E. coli* (since the RB69 genome is only 167,560 bp). As expected, the λZAP II vector alone yielded no plaques on *E. coli* groES42 ($<10^8$).

Our first surprise was that no plaques were found on *E. coli* groEL515. We had anticipated the putative λZAP II-31RB69 recombinant bacteriophage to grow on this host since bacteriophage λ propagates on *E. coli* groEL515 cells expressing Gp31T4 from a plasmid (Keppel *et al.* 2002). Our surprise continued when we unexpectedly found a high frequency of plaque-formers ($3 \times 10^{-3}$) on *E. coli* groEL44 lawns, similar to the frequency found on *E. coli* groES42. Subsequent plaque purification and retesting showed that these two groups of λZAP II recombinant bacteriophage behave similarly, i.e., all members of the two groups grow on both *E. coli* groES42 and *E. coli* groEL44, but none grow on *E. coli* groEL515. In addition, these λZAP II recombinant plaque-formers grow with an efficiency of 1.0 on our *E. coli* groES30 and groES619 mutants, but do not grow on the *E. coli* groEL140, groEL673, or groEL173 mutants ($<10^8$) (Table 2). Taken together, these observations suggested that, instead of isolating the putative 31RB69 cochaperone gene, we had identified a novel RB69 gene that modulates the host GroEL/GroES chaperone in an allele-specific manner.

*Identification of the gene responsible for bacteriophage growth on E. coli groE strains*

We proceeded to identify the putative RB69 gene(s) responsible for the seemingly allele-specific plating pattern of our λZAP II recombinant candidates. To determine the minimal region(s) of the RB69 genome represented by these clones, candidate inserts were sequenced from their ends (see
Comparison with the highly homologous T4 genome indicated that all candidates carried DNA from the region defined by the genes goF (aka comC-α), a putative antitermination factor (Takahashi and Yoshikawa 1979; Stitt et al. 1980), and gene 39, a component of T4 DNA topoisomerase (Huang 1986), located at approximately kbp 6 on the physical RB69 map (Figure 1). While most open reading frames in this region have homologs in T4, interestingly, and consistent with the genome plasticity of the bacteriophage T4 family, there is a unique gene, orf005c, that has been substituted for the T4 39.1 gene. A search of the T4 genomic DNA sequence showed that no homolog of orf005c exists, while a similar search of the RB69 genome indicated that it likewise has no homolog of 39.1.

Next, we reconstructed a λZAP II recombinant bacteriophage encompassing the common overlap among our λZAP II candidates, confirming that this approximately 3,100 bp RB69 DNA fragment is indeed responsible for allowing bacteriophage growth on the groEL44 and groES42 mutants. Subcloning specific RB69 open reading frames into the λZAP II vector allowed us to identify the 59 amino acid-coding 39.2 gene as solely responsible for the suppression phenotype (data not shown).

**Bacteriophage homologs of 39.2**

Because T4 is the prototype for this family of bacteriophages, we PCR-amplified and sequenced the equivalent region in T4. Near the end of the 39.2 gene, we noted several discrepancies with the published sequence of Huang (1986), which predicts a protein of 45 amino acid residues. Correction of these differences results in a predicted Gp39.2T4 protein more similar in sequence and length (58 amino acid residues) to that of Gp39.2RB69, with a homology of 91% and identity of
82% between the two (BLASTp). This is accurately reflected in the T4T sequence found at the GGC T4-like Genome website (http://phage.ggc.edu/).

Using gene 39.2T4-specific PCR primers, we amplified and sequenced the corresponding 39.2 homologs of the closely related bacteriophages T2, T6, K3 and OX2. As seen, these protein sequences are highly conserved with an overall pairwise identity above 80% (Figure 2). A striking feature in the alignment is the conservation of four cysteine residues (highlighted in red), typical of a zinc-binding motif. Subsequently, the DNA sequences of the more distantly related pseudo T-even bacteriophages RB43 and RB49 were completed and made available on the GGC T4-like Genome website. Although both bacteriophages RB43 and RB49 possess Gp39.2-coding genes, the auxiliary genes in their immediate neighborhood are by and large different from those shown in Figure 1 for T4 and RB69. Overall, the predicted Gp39.2RB43 and Gp39.2RB49 sequences are 30 - 40% identical to that of Gp39.2T4, as well as to each other (Figure 2). Interestingly, Gp39.2RB49 has only 3 of the highly conserved cysteine residues. However, the putative zinc-binding motif is retained since a histidine residue replaces the second cysteine (shown in red and indicated by an arrow at the bottom of Figure 2). Despite their relatively limited identity with Gp39.2T4, both Gp39.2RB43 and Gp39.2RB49 also suppress various GroE mutant phenotypes (see below and data not shown).

Construction of wild type and mutant 39.2 plasmids

The minimal 39.2 genes of bacteriophages RB69, T4, and RB49 were PCR-amplified and cloned under the tight regulation of the arabinose-inducible promoter PBAD (Guzman et al. 1995; Mayer 1995). Preliminary random PCR mutagenesis experiments with the minimal 39.2T4 gene suggested
that a mutation in one or more of the highly conserved cysteine residues (shown in Figure 2) abolishes Gp39.2 biological function. However, because these mutants had multiple changes, we constructed two 39.2 mutant clones by site-directed mutagenesis of plasmid pAC, carrying the minimal 39.2\textsuperscript{T4} gene. Changing the C13 codon to an alanine codon resulted in plasmid pDA1844. Similarly, changing the C31 and C34 codons to alanine codons resulted in a double mutant in plasmid pDA1846. Upon induction by arabinose, both mutant plasmids express levels of Gp39.2 comparable to the wild type clone (data not shown), although they completely lack activity in the biological assays shown below.

Suppression of bacteriophage resistance by Gp39.2

\textit{E. coli} wild type, \textit{groEL}44, \textit{groEL}515 and \textit{groES}42 cells were transformed with the plasmids expressing wild type and mutant Gp39.2\textsuperscript{T4}, as well as wild type Gp39.2\textsuperscript{RB69} and Gp39.2\textsuperscript{RB49} to test the effect of Gp39.2 on \textit{groE} mutant phenotypes. We first tested bacteriophage growth by spotting serial dilutions of a representative set of bacteriophages, namely \textit{λ}, T5, RB43, RB49, RB69, and T4, whose growth is blocked on the \textit{groEL}44 mutant. As a control, we used bacteriophage T4\textit{31} (L35I), whose mutant Gp31 (L35I) protein has a higher affinity for GroEL44, enabling the bacteriophage to grow normally on \textit{groEL}44 bacteria (Keppel \textit{et al.} 1990; Van Der Vies \textit{et al.} 1994; Richardson and Georgopoulos 1999; Richardson \textit{et al.} 1999). Clearly, only wild type Gp39.2\textsuperscript{T4} enables these bacteriophages to propagate on \textit{groEL}44 bacteria (Figure 3A), although both the wild type and C13A mutant Gp39.2 proteins are expressed at comparable levels. Analogous results were observed in certain other mutant \textit{groE} strains, and with plasmids expressing wild type Gp39.2\textsuperscript{RB69}, Gp39.2\textsuperscript{RB43}, and Gp39.2\textsuperscript{RB49} (data not shown). As expected from our original selection with the \textit{λZAP II-RB69} recombinant clones and the results shown in Table
2, expression of wild type Gp39.2\textsuperscript{T4} did not alter the bacteriophage plating properties of \textit{groEL515} mutant bacteria.

\textit{Suppression of temperature-sensitivity by Gp39.2}

The \textit{groEL44} strain does not grow at 42°. Thus, we asked whether expression of wild type Gp39.2\textsuperscript{T4} and Gp39.2\textsuperscript{RB49} can allow its growth at the nonpermissive temperature. As seen in Figure 3B, expression of wild type Gp39.2\textsuperscript{T4} and Gp39.2\textsuperscript{RB49}, but not Gp39.2\textsuperscript{T4} (C13A) or Gp39.2\textsuperscript{T4} (C31A C34A), partially restores growth of \textit{groEL44} mutant bacteria at 42°. Similarly, wild type Gp39.2\textsuperscript{RB69} and Gp39.2\textsuperscript{RB43} also partially suppress \textit{groEL44} growth at 42° (data not shown). It may be that Gp39.2 enables the mutant GroEL44 to fold, at least partially, all of its essential bacterial substrates at 42°.

\textit{Overexpression of Gp39.2 is toxic to E. coli growth}

We observed that expression of Gp39.2 can be toxic to \textit{E. coli} growth (Figure 4). By varying the amount of arabinose inducer present in the medium, we manipulated the intracellular Gp39.2 levels to show that toxicity depends on the \textit{groE} genetic background. Specifically, we found that \textit{groEL515} bacteria are more sensitive to Gp39.2 levels than wild type bacteria, which in turn are more sensitive than \textit{groEL44} bacteria. However, at very high levels, Gp39.2 is toxic even to \textit{groEL44} bacteria (data not shown). Thus, it appears that Gp39.2-mediated toxicity is directly or indirectly related to the function of the GroEL/GroES chaperone machine.

\textit{Gp39.2 expression enables folding of the UmuC protein}
As stated earlier, the GroEL/GroES chaperone machine is absolutely essential for *E. coli* viability because it is the only one that folds certain essential *E. coli* proteins (Houry *et al.* 1999; Kerner *et al.* 2005). Employing the observations of Donnelly and Walker (1989; 1992), we endeavored to demonstrate directly that Gp39.2 can play a role in the folding of a specific *E. coli* protein, UmuC. The UmuC and UmuD' (a cleaved product of UmuD) proteins make up DNA polymerase V (UmuD'_C; PolV) which is required for SOS-induced mutagenesis in *E. coli*. Specifically, Tang *et al.* (1999) showed that PolV is highly "error prone", capable of bypassing abasic lesions on UV-damaged DNA. Donnelly and Walker (1989; 1992) showed that the GroEL/GroES chaperone machine is required for PolV function, and that GroEL interacts directly with UmuC. Petit *et al.* (1994) further demonstrated in a purified protein system that GroEL/GroES enables UmuC to fold properly, leading to assembly of an active PolV enzyme.

Using Donnelly and Walker's procedure (1989), we showed that indeed *E. coli groEL44* mutant bacteria are deficient in UV-induced mutagenesis by monitoring the appearance of *E. coli* rifamycin-resistant (Rif^R^) mutants following UV-irradiation (data not shown). We then transformed the groEL44 bacteria with plasmid pAC encoding the wild type 39.2^T4^ gene, grew the resulting transformants in the presence or absence of arabinose, and followed the appearance of Rif^R^ mutants as a function of UV dose. As shown in Figure 5, expression of Gp39.2^T4^ substantially increases the appearance of Rif^R^ mutants following UV mutagenesis. We conclude that Gp39.2 directly or indirectly enables the mutant GroEL44 chaperone to help UmuC reach its native active state.
Proof of principle: Gp39.2 is essential for T4 survival on certain hosts

To determine if the seemingly nonessential 39.2 gene of T4 is in fact essential for bacteriophage growth on certain hosts, we constructed an isogenic T4 strain with an in-frame deletion of 39.2, using the insertion/substitution system of Selick et al. (1988). The T4 Δ39.2 mutant and its isogenic wild type parent were tested on our standard wild type E. coli K-12 host at various temperatures, and observed to plate equally well (Figure 6A and B). When tested on our groE mutant hosts, the two bacteriophages exhibited a differential growth pattern on E. coli groEL44. At the semi-permissive temperature of 30°, wild type T4 forms plaques, albeit small, while T4 Δ39.2 forms no plaques (Figure 6A). We found four additional strains and conditions under which the wild type T4 parental strain grows much better than T4 Δ39.2. The differential plating behavior of the two bacteriophages on E. coli groEL3388 at 39° is shown in Figure 6B. E. coli groEL3388, as well as the other three strains, carries the original groEL44 (E191G) mutation and an additional intragenic mutation that suppresses the temperature-sensitive phenotype of groEL44 bacteria, allowing them to form colonies at 43° (Klein and Georgopoulos, 2001). The intragenic mutation in groEL3388 results in the V190I substitution. The single groEL (V190I) mutation was also independently isolated in our laboratory as an extragenic suppressor of the groES619 temperature-sensitive phenotype at 42° (Zeilstra-Ryalls et al. 1994). Clearly, groEL3388 mutant bacteria completely block growth of the T4 Δ39.2 mutant. Although we have not yet analyzed the biochemical properties of the GroEL3388 protein, if T4 or its close relatives encounter a bacterial host in nature possessing a GroEL protein similar to GroEL3388, certainly those bacteriophages expressing Gp39.2 will be able to propagate on it.
Discussion

Because their short growth cycles (approx. 40 min) terminate upon host lysis, bacteriophages such as λ, T5 and T4 must complete their development in a timely fashion. In the case of T4, the most abundant protein produced by the bacteriophage is its major capsid subunit, Gp23, whose correct folding depends entirely on the host GroEL chaperone and the T4-encoded Gp31 cochaperone. Since both the groEL and groES genes are essential for E. coli viability (Fayet et al. 1989), any mutations that block bacteriophage growth cannot completely abolish the activities of these two genes. Through extensive genetic, biochemical and biophysical analyses, we showed that most of our groEL, groES and gene 31 mutants interfere with the normal GroEL/cochaperone cycle (Landry et al. 1993; Zeilstra-Ryalls et al. 1994; Landry et al. 1996; Richardson and Georgopoulos 1999; Richardson et al. 1999; Richardson 2000; Klein and Georgopoulos 2001; Shewmaker et al. 2004). Specifically, the groEL44, groES30, groES42, and groES619 mutations result in proteins with reduced affinity for the corresponding wild type cochaperone or GroEL partner. In contrast, the groEL515, groEL673, groEL173, and groEL (V190I) mutations result in mutant proteins that tend to prolong their interaction with the cochaperone partner. Based on these results and those reported here, we propose the following model to explain the suppression of groES and groEL mutations by Gp39.2. Figure 7 shows the backbone ribbon structure of a single GroEL subunit in either the "closed" (which binds substrate) or "open" (which binds GroEL's cochaperones) conformation (Braig et al. 1994; Xu et al. 1997; Sigler et al. 1998). The large difference between the two GroEL conformations is highlighted by the R322 and K178 residues, which form a salt-bridge in the "closed" conformation (likely stabilizing the structure), but which are found far apart in the "open" GroEL conformation. We suggest that, either directly or indirectly, Gp39.2 shifts
the equilibrium in favor of the GroEL "open" conformation. Gp39.2 can accomplish this by either destabilizing the "closed" or stabilizing the "open" GroEL conformation. This model is consistent with our bacteriophage plating results and Gp39.2 suppression of both host temperature-sensitivity and the UmuC defect in our groE mutant strains. It is also consistent with the observation that expression of Gp39.2 is best tolerated in the groEL44 host, followed by the wild type host, and is most toxic in the groEL515 host. We postulate that toxicity is observed in the groEL515 mutant because GroEL515 is held longer in the "open" state, rarely releasing the bound cochaperone heptamer, thus blocking the normal cycle of chaperone binding and timely release of substrate. Because Gp39.2 specifically suppresses weak GroEL/cochaperone interactions, we propose to rename its gene nwgI (normalizes weak GroE interactions).

Our observations also lead us to postulate that Gp39.2 acts through GroEL and not the cochaperone. The GroES protein of E. coli exhibits very low identity (<10%) with Gp31T4, Gp31RB43, and Gp31RB49 (aka CocO; Ang et al. 2001). In addition, Gp31T4, Gp31RB43, and Gp31RB49 are only 30-40% identical to one other. The fact that Gp39.2 expression suppresses all GroEL44 defects (which are due to GroEL44's weak interaction with its cochaperones; Richardson et al., 1999; Richardson, 2000; Shewmaker et al., 2004) regardless of the cochaperone, suggests that Gp39.2 engages directly or indirectly with GroEL to shift its equilibrium toward the "open" conformation, thus favoring interaction with any cochaperone. The isolation of E. coli mutants that tolerate high levels of Gp39.2 will perhaps help locate the putative GroEL-Gp39.2 interface.

Our failure to obtain plaque-formers from the λZAP II-RB69 library on our "high affinity" groEL515, groEL673, and groEL173 mutants suggests that either the RB69 genome does not
encode such a function or the function does not suppress these particular GroE mutant defects. However, based on our model of the biological role of Gp39.2, it would not be surprising to discover bacteriophages that do indeed encode a function opposite that of Gp39.2, i.e., a polypeptide that shifts the GroEL equilibrium towards the "closed" state. This putative function would be beneficial in hosts encoding a GroEL with a higher affinity for the bacteriophage-encoded Gp31, which would again slow down the chaperone cycle and timely maturation of Gp23, thus reducing the yield of bacteriophage progeny. We predict that such bacterial hosts should display a differential plating pattern between isogenic wild type T4 and T4 Δ39.2 bacteriophages. Thus far, we have not detected any such hosts among the various Enterobacteriaceae collections tested.

What is the origin of the bacteriophage 39.2 gene?

A search of the extant nucleotide sequence database shows that the Gp39.2 family of bacteriophage proteins is only encoded by those T4-like bacteriophages that can propagate on Enterobacteriaceae. Gp39.2 also belongs to the larger superfamily of so-called FmdB or CxxC_CxxC_SSSS proteins that contain a putative zinc finger domain found in a wide range of bacteria, but not Enterobacteriaceae. The superfamily is characterized by two CxxC motifs separated by approximately 17 amino acid residues. Consistent with the prediction that members of this superfamily likely bind zinc, we have shown that a monomer of Gp39.2T4 binds a molecule of zinc (unpublished data with Dr. Steve Alam, University of Utah). This suggests that zinc binding is necessary for the structure/function of Gp39.2 and helps explain why the Gp39.2T4 (C13A or C31A C34A) mutant proteins are inactive in in vivo biological experiments.
Curiously, no function has been assigned yet to FmdB, the founding member of this superfamily. The original fmdB gene, encoding a 112 amino acid polypeptide, was named thusly because it immediately follows the fmdA (formamidase) gene of Methylophilus methylotrophus (Wyborn et al. 1996), and is simply referred to as "putative FmdB regulatory protein" in the literature. Most non-bacteriophage FmdB family members carry the putative zinc-binding region at their amino-terminal end, and a 20-60 amino acid extension at their carboxyl ends, making them longer than Gp39.2 family members. There is a handful of recently identified and sequenced Myoviridae bacteriophages, very distant members of the T4-like superfamily, that all encode a bona fide fmdB gene of 81 amino acids in length. The original member of this small group is the Vi01 bacteriophage of Salmonella typhimurium (Pickard et al. 2010), whose putative FmdB product is 96% identical to the Salmonella enterica bacteriophage Det7 FmdB-like protein (Walter et al. 2008, S. Casjens, personal communication), and 94% identical to both the E. coli O157:H7 bacteriophage CBA120 (Kutter et al. 2011) and the Shigella boydii SboM-AG3 bacteriophage FmdB-like proteins (Anany et al. 2011).

Recently, Arbiol et al. (2010) provided evidence that at least two members of the greater T4-like family exchange genes by a novel mechanism of modular gene shuffling. Specifically, using bacteriophage RB43 and its very close relative coliphage phi1, Arbiol et al. (2010) identified small regulatory cassettes, termed PeSLs, which contain an early-type T4 promoter sequence and a transcription terminator. Modular shuffling of genes is likely mediated by recombination between PeSLs, and this genetic exchange likely proceeds through small circular DNA intermediates formed by the recombination event. Surprisingly, such small circular DNAs are detected not only in extracts of RB49/phi1-infected hosts but also in purified encapsidated virions. These novel and
exciting results offer an efficient mechanism for readily exchanging genes without destroying the parental DNA molecules in the process. For example, members of the Vi01 family of bacteriophages may have acquired a host *fmdB* gene(s). Subsequent co-infection of a common enterobacterial host with an ancestral T4-like bacteriophage may have resulted in exchange of the *fmdB* gene between the two bacteriophages, and eventual evolution to the present family of 39.2 genes.

The recent work of Yonesaki and colleagues exemplifies another potential role for apparently nonessential genes encoded by T4-like bacteriophages. By constructing an amber mutation in the T4 gene 61.5 (renamed *dmd*; encodes a 60 amino acid protein), Kai *et al.* (1996) showed that it is essential for growth only on certain *E. coli* hosts. Specifically, on these restrictive hosts in the absence of a functional 61.5 (*dmd*) gene, T4 late mRNAs are completely destabilized by RNase LS, thus blocking T4 late protein expression and growth. RNase LS is encoded by *rnlA*, a toxin gene whose product is inhibited by the product of the downstream antitoxin gene *rnlB* (Koga *et al.* 2011). The *rnlA-rnlB* toxin-antitoxin pair is located on the CP4-57 defective prophage in *E. coli* K-12 (Blattner *et al.* 1997). Thus, it appears that T4-like bacteriophages either have acquired and/or evolved the *dmd* gene in order to propagate on bacterial hosts possessing *rnlA*-like genes (apparently abundant in nature, judging from a BLASTp search for the *rnlA* gene).

*Closing the circle*

In the early 1970's in Geneva, Switzerland, one of us (C. G.) discussed with Dick Epstein whether one can isolate T4 mutants that propagate on *E. coli* K-12 strains but not on *E. coli* B, regardless of the host's ability to suppress nonsense mutations. Encouraged by Epstein, C. G. screened and
isolated two non-amber T4 mutants meeting these criteria, and showed that the mutations map in genes 8 and 53 (Georgopoulos et al. 1977). These two genes encode structural components of the wedges that make up the T4 baseplate (Yap et al. 2010). The simplest interpretation is that the mutations alter Gp8 or Gp53 in such a way that T4 cannot interact properly with the host receptor(s) necessary for proper T4 attachment and/or DNA injection. It is interesting to note that at least one of the original T4 "amber" mutants, HL626, originally thought to be in gene 60 (Yegian et al. 1971), is analogous to the mutants of Georgopoulos et al. (1977). These results exemplify the power of simple microbial genetic selections and screens to detect many classes of mutant phenotypes, provided they are set up appropriately. However, as seen with the 39.2 (nwgI) story described here, even what appears to be an obvious selection can lead to unexpected yet interesting results.

Acknowledgements

The authors are grateful to Dr. Jim Karam for the generous gift of the λZAP II-RB69 library, and thank Dick Epstein, Dominique Belin, France Keppel, Dana Carroll and Sherwood Casjens for useful discussions. This work was initiated at the University of Geneva, Switzerland, supported by FN-3100AO-114063. Currently, NIH R01 grant GM88207 supports this work.
FIGURE 1 Comparison of the RB69 and T4 g39 - cef regions. Arrows indicate open reading frames and their direction of transcription. goF is also known as comC-α (Takahashi and Yoshikawa 1979; Sanson and Uzan 1992). The RB69 orf005c gene is not a homolog of T4T 39.1. Only the beginning of gene 39 is shown. The scale above refers to the physical RB69 genome. The figure was drawn using information available on the GGC T4-like Genome website at http://phage.ggc.edu/.
Conserved putative zinc-finger residues are indicated in red. A recent search of 39.2 sequences in the GenBank Database suggests that Gp39.2 RB49 is so far unique in that it has a histidine rather than cysteine at the position indicated by the arrow. Asterisks indicate identity, colons indicate conservation among groups with strongly similar properties, and periods indicate conservation among groups with weakly similar properties (Larkin et al. 2007).
FIGURE 3  Wild type but not mutant Gp39.2 suppresses various GroE mutant phenotypes.

A. Suppression of bacteriophage resistance in groE mutants. Dilutions (10-fold) of GroE-dependent bacteriophages were spotted on lawns of E. coli groEL44 bacteria transformed with either pDA1844 (39.2 C13A) or plasmid pAC (39.2). The medium contained 0.01% arabinose and the plates were incubated overnight at 37°.

B. Suppression of the GroE temperature-sensitive phenotype by 39.2 expression. Dilutions (100-fold) of E. coli groEL44 transformed with pMPMK4 vector, pAC, pDA1844, pDA1846, or pAQ plasmids, respectively, were spotted on L-plates with or without 0.025% arabinose as indicated and incubated at 37° or 42° overnight. The nature of the plasmid-encoded protein is indicated along the top of the figures.
FIGURE 4 Expression of Gp39.2 is toxic for wild type *E. coli*. The B178 *groEL44* strain and its isogenic wild type parent were transformed with plasmids pAC, pMPMK4 and pDA1846. Cultures of these transformants were serially diluted 1:20 and spotted on an L-plate containing 50 µg/ml kanamycin and 0.05% arabinose. The plate was incubated at 37° overnight. The nature of the plasmid-encoded protein is indicated along the top of the figure.
FIGURE 5 Expression of T4 Gp39.2 restores UV-induced mutagenesis in *E. coli groEL44*. *E. coli groEL44* cells were transformed with plasmid pAC (wild type Gp39.2) and grown in the presence (black) or absence (red) of 0.02% arabinose. Experiment was performed twice. Frequency of mutagenesis to Rif\(^R\) was determined as described in Materials and Methods.
FIGURE 6 T4 Δ39.2 does not propagate on some *E. coli* strains. Serial dilutions (10-fold) of wild type T4 and T4 Δ39.2 were spotted on lawns of *E. coli* B178 strains, as indicated above and below each panel, respectively. A. Plates were incubated overnight at 30°. B. Plates were incubated overnight at 39°.
FIGURE 7 Model: Gp39.2 favors GroEL/cochaperone interaction by shifting the equilibrium of GroEL towards the "open state". To explain our genetic results, we propose that the Gp39.2 family of bacteriophage-encoded proteins directly or indirectly shifts the equilibrium of GroEL towards the "open" conformation, thus favoring binding of GroEL's cochaperones. Only one of the 7 GroEL subunits of the cis GroEL ring is shown here, based on the corresponding X-ray structure of Braig et al. (1994), Xu et al. (1997), and Sigler et al. (1998). The basic R322 and acidic E178 residues are highlighted in blue and red, respectively, to emphasize the massive en bloc rearrangements in the two structures (see text for details).
<table>
<thead>
<tr>
<th>Strains</th>
<th>Genotype or description</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>B178</td>
<td>K-12 W3110 galE sup°</td>
<td>Our collection</td>
</tr>
<tr>
<td>C600</td>
<td>K-12 thr-1 leuB6 lacY1 supE44 rbdD1 thi-1 tonA21 F⁻</td>
<td>Our collection</td>
</tr>
<tr>
<td></td>
<td>K-12 F⁻ mcrA Δ(mrr’hsdRMS mcrBC) Φ80lacZΔM15 ΔlacX74</td>
<td></td>
</tr>
<tr>
<td>DH10B</td>
<td>recA1 endA1 araD139 Δ(ara leu)7697 galU galK rpsL nupG; used for cloning purposes</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>CG217</td>
<td>B178 groES30 (A31V)</td>
<td>(Landry et al. 1993)</td>
</tr>
<tr>
<td>CG3009</td>
<td>B178 groEL515 (A383T)</td>
<td>(Zeilstra-Ryalls, et al. 1993)</td>
</tr>
<tr>
<td>CG3010</td>
<td>B178 groEL44 (E191G)</td>
<td>(Zeilstra-Ryalls et al. 1993)</td>
</tr>
<tr>
<td>CG3012</td>
<td>B178 groEL140 (S201F)</td>
<td>(Zeilstra-Ryalls, et al. 1993)</td>
</tr>
<tr>
<td>CG3015</td>
<td>B178 groEL673 (G173D)</td>
<td>(Zeilstra-Ryalls, et al. 1993)</td>
</tr>
<tr>
<td>CG3017</td>
<td>B178 groEL173 (Y199C)</td>
<td>(Revel et al. 1980; Richardson 2000)</td>
</tr>
<tr>
<td>CG3388</td>
<td>B178 groEL3388 (E191G V190I)</td>
<td>(Klein and Georgopoulos 2001)</td>
</tr>
<tr>
<td>DA1415</td>
<td>B178 groES42 (G23D)</td>
<td>(Landry et al. 1993)</td>
</tr>
</tbody>
</table>

**Bacteriophages**

- **λZAP II** accepts 0-10 kb inserts which can be excised *in vivo* via the Stratagene (Agilent)
<table>
<thead>
<tr>
<th>Phage Name</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ b2cl</td>
<td>clear-plaque former</td>
<td>our collection</td>
</tr>
<tr>
<td>T4Do</td>
<td>wild type T4</td>
<td>our collection</td>
</tr>
<tr>
<td>T4 K10</td>
<td>38amB262 51amS29 denAnd28 denB rIIB ΔrIIPT8</td>
<td>(Selick et al. 1988)</td>
</tr>
<tr>
<td>RB69</td>
<td>T4-like bacteriophage</td>
<td>(J. Karam)</td>
</tr>
<tr>
<td>RB49</td>
<td>T4-like bacteriophage</td>
<td>(H. Krisch)</td>
</tr>
<tr>
<td>RB43</td>
<td>T4-like bacteriophage</td>
<td>(H. Krisch)</td>
</tr>
<tr>
<td>K3</td>
<td>T4-like bacteriophage</td>
<td>(H. Krisch)</td>
</tr>
<tr>
<td>OX2</td>
<td>T4-like bacteriophage</td>
<td>(H. Krisch)</td>
</tr>
<tr>
<td>T2</td>
<td>T4-like bacteriophage</td>
<td>our collection</td>
</tr>
<tr>
<td>T5</td>
<td>T4-like bacteriophage</td>
<td>our collection</td>
</tr>
<tr>
<td>T6</td>
<td>T4-like bacteriophage</td>
<td>our collection</td>
</tr>
<tr>
<td>T4 Δ39.2</td>
<td>T4Do carrying deletion of 39.2 gene</td>
<td>this work</td>
</tr>
</tbody>
</table>

**Plasmids**

<table>
<thead>
<tr>
<th>Plasmid Code</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAA</td>
<td>pBSPLO(-) carrying regions flanking T4 Δ39.2</td>
<td>this work; (Selick et al. 1988)</td>
</tr>
<tr>
<td>pAC</td>
<td>pMPMK4 – 39.2 T4 KanR, high copy</td>
<td>this work; (Mayer 1995)</td>
</tr>
<tr>
<td>pDA1844</td>
<td>pAC – 39.2 T4 (C13A)</td>
<td>this work</td>
</tr>
<tr>
<td>pDA1846</td>
<td>pAC – 39.2 T4 (C31A C34A)</td>
<td>this work</td>
</tr>
<tr>
<td>pAQ</td>
<td>pMPMK4 - 39.2 RB49 KanR</td>
<td>this work; (Mayer 1995)</td>
</tr>
</tbody>
</table>
Table 2: Putative RB69 gene allows λ growth only on those *E. coli* groE mutants that result in a low-affinity GroEL/GroES interaction.

<table>
<thead>
<tr>
<th>Bacterial host</th>
<th>λZAP II vector</th>
<th>λZAP II-RB69 recombinant*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B178</td>
<td>+^a</td>
<td>+</td>
</tr>
<tr>
<td><em>groES42 (G23D)</em></td>
<td>-^b</td>
<td>+</td>
</tr>
<tr>
<td><em>groES619 (G24D)</em></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>groES30 (A31V)</em></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>groEL44 (E191G)</em></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>groEL140 (S201F)</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>groEL515 (A383T)</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>groEL673 (G173D)</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>groEL173 (Y199C)</em></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*The λZAP II-RB69 recombinant was selected for growth on the *E. coli* groEL44 mutant host. It is representative of all candidates isolated on either *E. coli* groEL44 or groES42.

^a "+" indicates growth of bacteriophage at an efficiency of 1.0.

^b "-" indicates no growth of bacteriophage (<10^-8).

^c The amino acid substitution resulting from each groE mutation is indicated in the parentheses following the allele designation.
Literature Cited


Georgopoulos, C., M. Georgiou, G. Selzer and H. Eisen, 1977 Bacteriophage T4 mutants which propagate on E. coli K12 but not on E. coli B. Experientia 33: 1157-1158.

Georgopoulos, C., 2006 Toothpicks, serendipity and the emergence of the Escherichia coli DnaK (Hsp70) and GroEL (Hsp60) chaperone machines. Genetics 174: 1699-1707.


