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Genomewide screens for *Escherichia coli* genes affecting growth of T7 bacteriophage

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Contributed by Charles C. Richardson, October 24, 2006 (sent for review October 6, 2006)

Use of bacteriophages as a therapy for bacterial infection has been attempted over the last century. Such an endeavor requires the elucidation of basic aspects of the host–virus interactions and the resistance mechanisms of the host. Two recently developed bacterial collections now enable a genomewide search of the genetic interactions between *Escherichia coli* and bacteriophages. We have screened >85% of the *E. coli* genes for their ability to inhibit growth of T7 phage and >90% of the host genes for their ability to be used by the virus. In addition to identifying all of the known interactions, several other interactions have been identified. *E. coli* CMP kinase is essential for T7 growth, whereas overexpression of the *E. coli* uridine/cytidine kinase inhibits T7 growth. Mutations in any one of nine genes that encode enzymes for the synthesis of the *E. coli* lipopolysaccharide receptor for T7 adsorption leads to T7 resistance. Selection of T7 phage that can recognize these altered receptors has enabled the construction of phage to which the host is 100-fold less resistant.

Bacteriophages have devised numerous strategies for growth in their bacterial host. In some instances they encode inhibitors of host proteins whose expression shortly after infection halt processes essential for host survival, substituting instead their own counter-part for phage growth. In other instances there is an acquisition of one or more host functions, thus minimizing the phage genome size. The identity of these phage–host relationships has, in the past, arisen largely from multiple independent studies. A genomewide approach for identifying these relationships would be valuable in that the ingenuity of the phage in acquiring host functions leads to unexpected, but extremely interesting, biochemical and genetic strategies. An understanding of these phage–host relationships at the molecular level could, in turn, lead to the construction of phages that depend less on certain properties of the host and consequently would be more virulent. Likewise, the identification of host proteins that hinder phage infection and growth would be instructive in designing phages that could overcome them. Such phages deplete their host with minimal acquired resistance and may thus serve as an attractive alternative to antibiotic treatment. It would be virtually impossible to isolate phages in nature to which no bacterial resistance readily evolves. Such phages deplete their host and thus eliminate their sole mechanism of propagation.

Bacteriophage T7 and its host, *Escherichia coli*, provide a model for systematically studying host–virus interactions. Their genetics have been studied extensively, and the functions of >50% of the 56 genes of T7 and 4,453 genes of *E. coli* have been elucidated. The recent development of two *E. coli* collections has enabled the systematic search for interactions between *E. coli* and T7 phage. One, the “Keio collection” (1) contains single-gene knockout mutants of all of the nonessential genes in *E. coli* K-12. The other, the “ASKA library” (2) contains *E. coli* cells expressing most of the *E. coli* genes from cloned plasmids.

A single T7 phage produces >100 progeny after infection of *E. coli*. The phage inhibits some *E. coli* proteins that interfere with its replication. For example, T7 produces a protein, gp0.3, that binds to and inhibits the *E. coli* type I restriction system (3). Other examples include the inhibition of the dGTP triphosphatase by the phage gp1.2 (4, 5) and the inhibition of the *E. coli* RNA polymerase by gp2 and gp0.7 (6–8). In this article we use the ASKA library to carry out a systematic screen to identify other *E. coli* genes that interfere with T7 growth.

A screen for genes that are essential for the intracellular growth of T7 was carried out by Chamberlin (9). One class of genes that he found, designated *tsnC*, consists of mutants in the *tsnA* gene (10); the product of this gene, thioredoxin, acts as the processivity factor for T7 DNA polymerase (11). In this earlier study other essential genes may well have been missing in the mutant population because of nonrandom distribution of the induced mutations and the different growth characteristics of the mutated bacteria. In addition, Chamberlin chose not to characterize the *tsnA* group of genes that are essential for adsorption of T7 to the cell surface of *E. coli*. Identification of the biochemical defects in those mutants identified in this early screen was made difficult by the fact that proteins such as thioredoxin had not yet been mapped to a specific gene. A thorough and complete systematic screen covering virtually the entire nonessential *E. coli* genes has now become possible because of the development of the Keio collection (1).

Using the ASKA library and the Keio collection (1, 2), we have carried out two genetic screens, one for finding host genes that inhibit T7 growth and another for identifying host genes that are required for T7 growth. In addition to finding all of the expected genes in our screens, we have identified several genes that were not known to be involved in T7 growth. We have used our findings to construct, by genetic selections, T7 phage that are more resistant to mutations in the host than is WT T7 phage.

Results and Discussion

**Screen for *E. coli* Genes that Interfere with T7 Phage Growth.** We used the ASKA library to screen for host genes interfering with T7 growth (Fig. 1). The ASKA library contains 85% of the *E. coli* ORFs under the isopropyl β-D-thiogalactoside (IPTG)-inducible *tac* promoter on high-copy plasmids (2). We plated the ASKA library on agar that contains T7 phage and IPTG, as described in *Materials and Methods*. Three different T7 phages, D104/LG37, LG30, and HS33, were used for three independent screens; each was deleted for a nonessential region of T7 (see *Materials and Methods* for genes deleted). Using these phages enables the identification of genes in the host that can be overcome by phage-encoded genes.

After overnight incubation, surviving colonies were identified. We assume these colonies are resistant to T7 because the gene encoded by the plasmid confers T7 resistance. Another possibility is that these colonies acquired a secondary mutation that renders them resistant. To distinguish between these two possibilities,
plasmids from the original plates that had given rise to these surviving colonies were extracted and retransformed into a fresh stock of cells. After this procedure, the phenotype was tested again by plating on T7-containing agar. We have identified four genes that when overexpressed inhibit T7 growth on E. coli. The efficiency of plating (EOP) of the T7 deletion mutants on these strains as compared with a control is indicated in Table 1.

As expected, the two genes that are known to inhibit T7 growth when overexpressed, hsdR and dgt (4, 5, 12), were identified when T7 encompassing deletions in genes 0.3 and 1.2, respectively, was used (Table 1). hsdR constitutes the restriction subunit of EcoK1, which, as mentioned above, is inhibited by gp0.3. dgt encodes a nucleotide triphosphatase that hydrolyzes dGTP. The dGTPase is inhibited by T7 gp1.2, and T7 nucleotide triphosphatase that hydrolyzes dGTP. The dGTPase is inhibited by T7 gp1.2, and T7 nucleotide triphosphatase that hydrolyzes dGTP.

The third gene identified in this screen was rcsA. Overexpression of a truncated gp0.7 (T7-JS62a) lyses udk-overexpressing cells significantly slower than it lysed the control. WT T7 lyses the other tested T7 deletion mutants, which express gp0.7 normally, lysed E. coli expressing udk as rapidly as did the WT T7 (data not shown). We therefore conclude that gp0.7 overcomes the effect of udk overexpression. Gp0.7 is a protein kinase that is known to phosphorylate E. coli RNA polymerase (17, 18), but the specific mechanism by which gp0.7 overcomes the inhibition of udk overexpression is unknown. We speculate that overexpression of udk interferes with the inhibition of the host RNA polymerase by T7. A T7 suppressor to the phenotype has been mapped to gene 2, and

Table 1. Genes identified as inhibitors of T7 growth

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene product</th>
<th>T7 gene that overcomes inhibition</th>
<th>EOP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>dgt</td>
<td>dGTP triphosphatase</td>
<td>1.2</td>
<td>&lt;10^{-2}</td>
</tr>
<tr>
<td>hsdR</td>
<td>Subunit of the EcoK1 restriction enzyme</td>
<td>0.3</td>
<td>0.06</td>
</tr>
<tr>
<td>rcsA</td>
<td>Activator of capsule synthesis genes</td>
<td>None</td>
<td>0.9†</td>
</tr>
<tr>
<td>udk</td>
<td>Uridine/cytidine kinase</td>
<td>0.7</td>
<td>&lt;10^{-2}</td>
</tr>
</tbody>
</table>

*EOP of T7 D104/LG37 relative to its EOP on WT E. coli.
†Turbid plaques.

Fig. 1. Schematic presentation of the screening procedure. Cells were transferred from the E. coli collections onto agar plates layered with T7 phage and grown overnight. (Left) The identification of E. coli overexpressing the dGTP triphosphatase (E. coli/dgt). dgt overexpression inhibits T7 growth, but can be overcome by the activity of gp1.2 (5). A phage deleted for gene 1.2 would identify E. coli/dgt, whereas a screen with the WT T7 would not. (Right) A similar procedure for screening the Keio collection. Use of deletion T7 mutants in this procedure can lead to identification of genes in E. coli that are homologues of those deleted in the T7 phage.
gp2, similarly to gp0.7, plays a role in host RNA polymerase inhibition.

Thus the genomewide screen not only identified all of the known genes that interfere with T7 phage growth but also identified two additional genes, rcsA and udk. T7 phage has evolved proteins that overcome the effect of three of these four genes. It is theoretically possible to allow T7 to overcome the effect of the gene activating capsule expression (rcsA) by introducing a gene product that hydrolyzes the colanic acid capsule. Indeed, a similar approach has been successfully demonstrated on E. coli K1 by using a T7 phage encoding an enzyme that hydrolyzes the K1 capsule (15).

Screen for E. coli Genes That Are Essential for T7 Phage Growth. To identify host genes that are required for T7 growth, we carried out a screen using the Keio collection (1) (Fig. 1). The collection was plated on an agar layer of T7 phage, and colonies that survived were further screened for the effect of the deleted genes on T7 growth. In this collection, >90% of E. coli genes could be individually deleted, indicating that under rich medium growth conditions they are nonessential. Three different T7 phage containing deletions in a number of T7 nonessential genes have been used for three independent screens. The use of T7 deletion mutants allows us to also detect host genes that are essential for T7 growth only when a T7 gene is lacking. For example, gene 1.3 encodes for a ligase that is nonessential for growing on WT host, because the phage can use the host functional ligase. However, gene 1.3 becomes essential for growing on a host in which ligase is not functional at the time of infection (19). Thus, using T7Δ1.3 one would theoretically identify a host lacking the ligase gene. Practically, because ligase is essential for E. coli, it is not represented in the library, and therefore it would not be identified in our screen.

Table 2 summarizes the mutants that were identified after the screening procedure. We have validated the phenotype by measurement of the EOP on the identified clones and complementation of the replaced gene with a plasmid encoding for this gene. The genes identified can be classified into three groups: (i) trxA, (ii) cmk, and (iii) LPS biosynthesis genes. All bacteria deleted in these genes showed a significantly lower EOP as compared with the control bacteria. In addition, all of the identified deletion mutants were complemented by a plasmid encoding the respective deleted gene.

The trxA gene, as expected, was identified in this screen as being essential for T7 growth. The trxA gene encodes thioredoxin, the processivity factor for T7 DNA polymerase (11).

A second essential gene for T7 growth is cmk. cmk encodes for CMP kinase that catalyzes the conversion of CMP and dCMP into CDP and dCDP, respectively, using ATP as the phosphate donor (20). This gene is not essential for E. coli but in its absence bacteria replicate at a slower rate (21). T7 phage, upon infection of E. coli, express an exonuclease (gp6) and an endonuclease (gp3) that hydrolyze the host chromosome to deoxyribonucleoside 5’-monophosphates. The resulting nucleotides are then presumably converted to the corresponding nucleoside diphosphates and triphosphates, the latter being the substrates for T7 DNA polymerase. Earlier studies have shown that >80% of the nucleotides found in T7 phage are derived from the breakdown of host DNA (22). The dependency of T7 on this salvage pathway most likely leads to a requirement for nucleoside 5’-monophosphate kinases for each of the four nucleosides. Other nucleoside monophosphate kinases (encoded by adk, tmk, gmk, and pyrH) are essential genes for E. coli and therefore would not arise in this screen. Precisely why E. coli also does not depend on CMP kinase is not clear but it may reflect the presence of other pathways that give rise to dCDP because cytosine nucleotides originate by amination of UTP, yet the conversion to deoxynucleotides by ribonucleotide reductase occurs only at the level of the nucleoside diphosphate (21).

The other genes identified to be essential for T7 infection encode for nine enzymes that are involved in the biosynthesis of the outer membrane LPS. It is known that LPS recognition by the T7 receptor is essential for phage adsorption to the host, but the exact sugar moiety to which the phage binds is not known (22). The core structure of E. coli K-12 LPS is depicted in cartoon form in Fig. 3, along with mutant LPS structures (chemotypes). The LPS core is composed of lipid A, which is embedded in the outer membrane and to which a backbone of Kdo, heptoses, and glucoses are attached. The genes gnhA, gnhB, gnhD, and gnhE encode for enzymes in the heptose biosynthesis pathway. Absence of any of these genes results in a type Re LPS, having only the Kdo sugars, as shown in Fig. 3 [an exception being gnhB, which has a partial Re phenotype (23) and may explain the different EOP measured on this strain compared with the other Re mutants]. Other genes that participate in enzymatic steps of the LPS core biosynthesis are: waaA, waaC, waaF, waaG, galU, waaO, waaR, and waaK, as depicted (24) [galU exhibits a leaky LPS phenotype (25), which may account for the differences in the EOPs between galU and waaG]. All of these genes except waaA, waaO, and waaK were identified in our screen. The waaA and waaU genes, catalyzing the addition of the Kdos and the terminal heptose, respectively, could not be identified because they are not represented in the Keio collection (1). The waaO was not identified in the screen and was shown to be not essential to T7 infection. The fact that addition of a single

Table 2. Genes identified as essential for T7 growth

| Gene     | Gene product* | EOP after complementation \\ | EOP |\
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>trxA</td>
<td>Thioredoxin A</td>
<td>&lt;10⁻²</td>
</tr>
<tr>
<td>cmk</td>
<td>CMP/dCMP kinase</td>
<td>0.05⁺</td>
</tr>
<tr>
<td>gnhA</td>
<td>LPS biosynthesis enzyme</td>
<td>&lt;10⁻²</td>
</tr>
<tr>
<td>gnhB</td>
<td>LPS biosynthesis enzyme</td>
<td>0.30⁺</td>
</tr>
<tr>
<td>waaC</td>
<td>LPS biosynthesis enzyme</td>
<td>&lt;10⁻²</td>
</tr>
<tr>
<td>gnhD</td>
<td>LPS biosynthesis enzyme</td>
<td>&lt;10⁻²</td>
</tr>
<tr>
<td>gnhE</td>
<td>LPS biosynthesis enzyme</td>
<td>0.01</td>
</tr>
<tr>
<td>waaF</td>
<td>LPS biosynthesis enzyme</td>
<td>0.23⁺</td>
</tr>
<tr>
<td>waaG</td>
<td>LPS biosynthesis enzyme</td>
<td>0.01</td>
</tr>
<tr>
<td>galU</td>
<td>LPS biosynthesis enzyme</td>
<td>0.29⁺</td>
</tr>
<tr>
<td>waaR</td>
<td>LPS biosynthesis enzyme</td>
<td>&lt;10⁻²</td>
</tr>
</tbody>
</table>

*For detailed description of gene product, see Fig. 3.

¹EOP of WT T7 relative to its EOP on WT E. coli.

²Small/turbid plaques.

³EOP of WT T7 on indicated host harboring a plasmid, which encodes the deleted gene, relative to the EOP on WT E. coli.

Figure 2. Growth curves of E. coli udk- or mock-overexpressing cells infected with WT T7 or T7-J562a. T7-J562a contains a nonsense mutation in gene 0.7 at codon 126. E. coli cells harboring a plasmid encoding the udk gene or thr5 gene (mock) were grown to midlog phase, induced, and infected by the designated T7 phage. OD₆₀₀ measurements were taken every 5 min as described in Materials and Methods.
glucose to the two core heptoses, as is the case in waaO host, allows T7 to infect the host implies that the first glucose of the LPS core is a binding site for T7. However, when one or more glucose residues are attached to this glucose, the binding site is probably hindered, and therefore either the penultimate glucose or the terminal heptose of the K-12 LPS backbone or both become essential for T7 infection. We also conclude that the side chains (not shown in Fig. 3) of the LPS are not important for T7 recognition, because the genes catalyzing these steps were not identified in the screen. Indeed, WT T7 showed similar EOP when tested individually on waaL, waaP, waaQ, waaS, waaY, and waaZ hosts (lacking genes that catalyze LPS side-chain additions/modifications), as compared with its EOP on WT E. coli. The EOP of WT T7 on waaB host (lacking a gene that catalyzes LPS side-chain addition) was slightly reduced (≈0.6) relative to the EOP of WT E. coli, but the plaques had normal morphology. Although this study identifies the complete LPS genotype associated with T7 resistance, others have also isolated E. coli K-12 mutants resistant to T7 (26). They postulated that the LPS moieties that form the binding site for T7 are the inner core heptoses, based on differential recognition patterns of various phages. Indeed, the waaF mutant, having Rd2-type LPS with only one heptose protruding can support phage infection to some extent, with a relatively high EOP of ≈0.2, as compared with the other mutants. This finding explains why the heptoses might have been considered the binding site for T7 receptor. However, our study implies that other moieties of the LPS, namely the first glucose, and the terminal glucose/heptose may play a more significant role in receptor recognition.

Overall, we have identified one gene previously known to be essential for T7 growth and 10 additional genes essential for T7 phage growth. The findings can be used to construct phages that are independent of nonessential host genes and consequently more virulent to the host. One approach toward achieving this goal is to insert the trxA and cmk genes into the T7 genome, thus reducing T7 dependency on host genes. Indeed, trxA has been inserted into many strains of T7 and these phages grow independently of host trxA expression. In addition, we have successfully introduced a cmk gene into T7 to select for recombinant phages, which do not depend on cmk expression by the host. These results show that (i) cmk can be introduced to T7 and thus render the phages independent of host cmk, and (ii) cmk can be used as a marker to select for T7 encoding it, in a similar way that trxA has been used (quite efficiently, as described in Materials and Methods). The availability of a second genetic marker simplifies genetic manipulations, for example, in constructing double deletion T7 mutants.

**Construction of T7 Phages That Are More Virulent Than WT T7.** From the data presented above it is clear that resistance to infection by phage T7 is most likely to arise by mutations in one or more of the 10 genes in the pathway leading to synthesis of the T7 receptor, the LPS. Therefore, we were curious to see whether we could isolate more virulent T7 phages, more virulent in the sense that less host resistance develops, by selecting for phages that can infect E. coli having alterations in the LPS that render them resistant to infection. Following the selection procedure in Materials and Methods we first selected for T7 phages that could infect E. coli having the shortest LPS (Re LPS in Fig. 3). As shown in Table 3, the selected mutant, T7-Re, can infect a host with the Re LPS as efficiently as WT T7 can infect WT E. coli. Interestingly, T7-Re can still infect WT E. coli with high efficiency. In addition, it can now infect E. coli mutants having defects in the LPS that give rise to LPS Rd1 and at a lower efficiency also to Rb (waaG and waaR, respectively). Inasmuch as T7-Re remained unable to infect strains with the Rd2 and Rb LPS (waaF and waaR) we selected for T7-Re phages that could grow on a Rb LPS (waaR host). The resulting mutant phage, T7-ReRb, gained the ability to infect waaR hosts and surprisingly retained its ability to grow on all of the other LPS mutants that T7-Re could infect (Table 3). In fact, its ability to grow on E. coli waaR was enhanced. The final selection was for a T7-ReRb phage that could grow on the one remaining LPS Rd2 mutant, E. coli waaF (Table 3). In this manner, as shown in Table 3, we constructed a mutant T7 phage that is essentially independent of the LPS receptor.

**Table 3. Plating efficiency of the indicated phage on LPS mutants**

<table>
<thead>
<tr>
<th>Phage</th>
<th>E. coli strain (LPS structure)</th>
<th>K12</th>
<th>waaR (Rb)</th>
<th>waaO (Rc)</th>
<th>waaG (Rd1)</th>
<th>waaF (Rd2)</th>
<th>waaC (Re)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT T7</td>
<td></td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T7-Re</td>
<td></td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>T7-ReRb</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>T7-ReRd2Rb</td>
<td></td>
<td>++</td>
<td>++</td>
<td>+++*</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

*EOP of indicated phage on indicated host, relative to its EOP on WT E. coli: ++ indicates 0.7 ≤ EOP; + + indicates 0.3 ≤ EOP < 0.7; * indicates 0.1 ≤ EOP < 0.3; – indicates EOP < 0.1.

+*EOP > 10.

---

**Fig. 3.** Cartoon of the LPS structures on E. coli mutants. The biosynthesis pathway of the core LPS formation is indicated above the structures. Boxed genes are not represented in the Keio collection. Parenthesized genes display partial phenotype. The nomenclature of the LPS chemotypes was reviewed by Raetz (28).
We determined the frequency of host resistance to WT T7, T7-Re, T7-ReRb, and T7-ReRd2Rb. These four phages were used to individually infect 2 × 10^9 or 10^7 WT E. coli at a multiplicity of infection (MOI) of 0.5 and 10, respectively. As expected, host resistance to WT T7 was significantly higher (~10,000 and ~800 resistant colonies using MOI of 0.5 and 10, respectively) than host resistance to T7-Re (65 and 8 resistant colonies using MOI of 0.5 and 10, respectively), T7-ReRb (44 and 7 resistant colonies using MOI of 0.5 and 10, respectively), and T7-ReRd2Rb (134 and 31 resistant colonies using MOI of 0.5 and 10, respectively). Thus, T7-Re and T7-ReRb are at least 100-fold more virulent than WT T7. The remaining resistant cells most likely contain mutations in genes not related to the LPS biosynthesis pathway. Fig. 4 shows the colonies arising after infection by the indicated phage at an MOI of 0.5. The colonies on the WT T7 plate are mostly small and not mucoid, whereas the colonies on the mutant phage are mostly large and mucoid. This result suggests that up-regulation of the rcsA gene (Table 1), or other mutations leading to the increased capsule formation, can be the factor bestowing phage resistance in these colonies.

In this article, we highlight the importance of developing screens to identify host–virus interactions and their subsequent possible use for boosting phage therapy. The proofs of principles presented here for the relatively simple T7 and its host E. coli might pave the way toward similar use with other phages. When a collection of knockout mice becomes available (27), the principles presented here could be applied to identify host genes essential to eukaryotic viruses. The identity of genes that are essential for virus replication could lead to strategies that prevent or hinder viral growth.

Materials and Methods

Materials. LB broth, LB agar, granulated agar, and kanamycin were purchased from EMD (San Diego, CA). Chloramphenicol was purchased from American Bioanalytical (Natick, MA).

Bacterial Strains and Phage. Bacterial strains were provided by Hirotada Mori (Nara Institute of Science and Technology, Nara, Japan). The Keio collection was constructed on E. coli K-12 BW25113: 

 materials correspond to T7 genome, GenBank accession no. NC_001604.  

Table 4. Strains used for screening the ASKA library and Keio collection

<table>
<thead>
<tr>
<th>T7 phage strain</th>
<th>Deleted segment*</th>
<th>Deleted genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>D104/LG37</td>
<td>579–2736; 5847–7762</td>
<td>0.3, 0.4, 0.5, 0.6, 0.7, 1.1, 1.2, 1.3, 1.4</td>
</tr>
<tr>
<td>LG30</td>
<td>6575–8434</td>
<td>1.3, 1.4, 1.5, 1.6 (1.7 truncated at 5’ end)</td>
</tr>
<tr>
<td>H533</td>
<td>13279–14284</td>
<td>4.3, 4.5, 4.7</td>
</tr>
</tbody>
</table>

*Base pair numbers correspond to T7 genome, GenBank accession no. NC_001604.
containing only one or several colonies proved to be not reproducable and were treated as false positives.

The Keio collection was replica-plated on large LB-kanamycin-agar Petri dishes overlaid with soft agar containing phage. The plates were incubated at 37°C for 20 h. Surviving colonies were restested by using an independent gene-replacement mutant colony provided in the Keio collection. Colonies that consistently showed resistance to T7 growth were chosen for further analysis. As with the ASKA collection screen, only bacteria that showed a continuous lawn of bacteria on the replica-plated spot proved to be actual T7-resistant colonies.

**EOP Assays.** Selected colonies that were scored positive in the ASKA screen were grown overnight, and 1 ml of the culture was mixed with 100 pfu WT T7 in soft agar. The mixture was overlaid on LB plates, and after 15 h plaques were counted. The pfu were normalized to the pfu obtained on a mock-transformed E. coli (E. coli with a plasmid expressing a gene that does not affect T7 growth, thrS).

A similar assay was carried out to determine the EOP by the colonies that scored positive in the Keio screen. In this case, the pfu counts were normalized to pfu counts obtained on a mock knockout strain (E. coli with a kanamycin insertion in a gene that does not affect T7 growth, yhdQ).

**Complementation Assays.** To validate the results obtained with the Keio collection, plasmids encoding the knocked-out genes were extracted from colonies in the ASKA library and transformed to their respective strains in the Keio collection. These transformed strains were checked for their EOP as described above.

**Kinetic Lysis Assays.** Midlog phase bacteria expressing either udk or the control thrS were grown in the presence of 1 mM IPTG for 45 min. A total of 180 μl of the cell suspension was then dispensed to wells in a 96-well plate. Twenty microliters of WT T7 and T7 phage mutants was added to the cell suspension at an MOI of 10⁻⁷ to 10⁻⁴ (similar MOI of each phage was used on the udk- and thrS-overexpressing hosts). The plate was incubated for 15 h in a Spectramax M5 spectrophotometer ( Molecular Devices, Sunnyvale, CA) at 37°C, and every 5 min the plate was shaken and OD₆₀₀ was measured. Differences similar to those shown in Fig. 2 were also observed when using higher MOIs.

**Using cmk as a Selection Marker for cmk-Encoding T7 Phage.** Δ₅::cmk phage was generated by recombination between WT T7 phage and a pGp5-cmk plasmid encoding the cmk gene flanked by sequences upstream (400 bp) and downstream (200 bp) of gene 5. The pGp5-cmk was generated by using multistep PCR and a TOPO cloning kit (Invitrogen, Carlsbad, CA). Liquid culture of DH5α cells transformed with pGp5-cmk plasmid at an OD₆₀₀ = 0.3 was infected with T7 WT phage at a MOI of 0.1 and grown until complete lysis. The lysate was plated on DH5α cells transformed with pGp5 plasmid, encoding WT gp5. Forty-eight large plaques were transferred to a microtiter plate and replica-plated on E. coli strains DH5α, K-12 Δcmk, and K-12Δcmk/pGp5. All 48 plaques exhibited a phenotype, expected for Δ₅::cmk T7 phage, i.e., negative for growth on DH5α and K-12 Δcmk (because of lack of gene 5), and positive for growth on K-12 Δcmk/pGp5, where gene 5 is supplied from the pGp5 plasmid and cmk is supplied by the recombinant phage. Three plaques were randomly picked and the gene replacement was confirmed by direct sequencing of the genomic DNA from the recombinant phage.

**Selection and Sequencing of Phage Mutants that Infect T7-Resistant Colonies.** To obtain T7-Rec, T7-RecRb, and T7-RecRd2Rb, selection of plaques that form on strains that have truncated LPS was sequentially carried out. T7-RecRb was sequenced from base pairs 17649–39937. The identified mutations map to genes 11, 12, and 17: gp11-M6V, gp12-D181G, gp12-P694T, gp17-N501H, and gp17-R542H. The other two mutants and the WT T7 were sequenced in genes 11, 12, and 17 only. T7-Rec contains the following substitutions: gp11-M6V, gp12-D181G, and gp17-N501H. T7-RecRd2Rb contains the following substitutions: gp11-M6V, gp12-D181G, gp12-P694T, gp17-N501H, gp17-R542H, and gp17-V544A. The WT T7 used in our laboratory is identical in these three genes to the published sequence (GenBank accession no. NC_001604) in all encoded residues except gp12-S694P.

**Determining the Number of E. coli Colonies that Survive T7 Infection.** The virulence of T7-Rec, T7-RecRb, T7-RecRd2Rb, and WT T7 phage was determined as follows. Soft agar containing 5 × 10⁶ cfu or 10⁷ cfu of WT E. coli was mixed with each phage at an MOI of 0.5 and 10. The suspension was overlaid on LB plate and incubated at 30°C. The next day, surviving bacteria were counted.

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The published sequence (GenBank accession no. NC_001604) is supplied with E. coli K-12 cmk in all 5::cmk regions.