Population Fitness and the Regulation of Escherichia coli Genes by Bacterial Viruses

Ying Chen, Ido Golding, Satoshi Sawai, Ling Guo, Edward C. Cox*

Department of Molecular Biology, Princeton University, Princeton, New Jersey, United States of America

Temperate bacteriophage parasitize their host by integrating into the host genome where they provide additional genetic information that confers higher fitness on the host bacterium by protecting it against invasion by other bacteriophage, by increasing serum resistance, and by coding for toxins and adhesion factors that help the parasitized bacterium invade or evade its host. Here we ask if a temperate phage can also regulate host genes. We find several different host functions that are down-regulated in lysogens. The pckA gene, required for gluconeogenesis in all living systems, is regulated directly by the principal repressor of many different temperate prophage, the cl protein. cl binds to the regulatory region of pckA, thereby shutting down pckA transcription. The pckA regulatory region has target sequences for many other temperate phage repressors, and thus we suggest that down-regulation of the host pckA pathway increases lysogen fitness by lowering the growth rate of lysogens in energy-poor environments, perhaps as an adaptive response to the host predation system or as an aspect of lysogeny that must be offset by down-regulating pckA.

Introduction

A central question in the biology of host–parasite interactions is how a balance between the costs and benefits to both is achieved. If the burden to the host is too high, the parasite will go extinct, and for this reason it is often postulated that parasites confer some benefit upon their hosts, thereby arriving at an equilibrium in the competition between parasite-free and infected host populations.

Bacterial viruses are parasitic. Roughly speaking, they either invade and kill the host or they invade and lie dormant, either integrated into the host genome or outside it as extrachromosomal elements [1]. Phage that can exist in a dormant state are called temperate phage, and bacteria carrying temperate phage are said to be lysogenic. In the lysogenic state, viral functions needed for replication and packaging are shut down by a phage-encoded repressor. Occasionally, a temperate phage genome escapes repression, the virus begins to replicate, and soon the host cell lyses, producing a new generation of viral particles (Figure 1A). The lysogenic state thus imposes a cost on the bacterium because every so often the viral genome (prophage) replicates and kills the host. This selective disadvantage is offset, however, in several important ways. Because prophage produce a repressor that keeps the prophage genes from being expressed, the host bacterium enjoys immunity from lytic infection by temperate family members. A second, and different, mechanism confers immunity to lytic phage, those phage that cannot exist in the host as prophage [2,3]. Temperate phage may also confer fitness on a host by coding for genes that enhance host virulence and resistance to the immune system [4], of which there are dozens of examples: the Shiga toxin produced by some strains of Escherichia coli; the β toxin produced by Corynebacterium diphtheriae, the causative agent of diphtheria; endotoxin production by Clostridium botulinum; staphylococcal endotoxins; and cholera toxin produced by Vibrio cholerae, to name a few. Then too, prophage often code for functions that allow the lysogen to successfully colonize the animal host [4], and in general, temperate phage increase horizontal gene flow in microbial populations [5]. Thus there are several advantages to being a lysogen, and at least one big disadvantage: Occasionally the prophage replicates and kills the host.

Of the many prophage that litter bacterial genomes, the best studied is λ. When it recombines into the E. coli chromosome, phage multiplication is shut down by the phage-encoded repressor, cl, a critical element of the genetic switch. The continuous low-level production of cl protects the host against further infection by extracellular λ, while also regulating the levels of cl synthesis intracellularly (reviewed in [6]). The phage also codes for genes required for replication, maintenance, integration, and escape from the host cytoplasm, as well as a series of genes not required for growth in the laboratory (Figure 1B).

Most cl genes are repressed by cl, but there are several whose transcription is constitutive, their expression either dependent or independent of cl control. Two, the products of the rexA and rexB genes, exclude productive infection by the unrelated lytic bacteriophage T2, T4, and T6 [2]. The Rex proteins have also been reported to increase the advantage of lysogens in competition experiments [7], but there are conflicting results in the literature on this point [8]. Two other proteins, Bor and Lom, are found in the host outer membrane, and Bor lysogens are resistant to guinea pig serum [9,10]. Each of the above examples illustrates how prophage-

Received March 2, 2005; Accepted April 27, 2005; Published June 21, 2005
DOI: 10.1371/journal.pbio.0030229

Copyright: © 2005 Chen et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abbreviation: bp, base pair

*To whom correspondence should be addressed. E-mail: ecox@princeton.edu
encoded λ genes increase lysogen fitness by coding for a protein that protects the host from invaders or from the humoral system.

Here we ask if the phage repressor directly or indirectly regulates host genes. There is indirect evidence that a streptococcal temperate phage may regulate a bacterial gene that protects cells against phagocytosis [11], but there have been no systematic studies on this subject. By surveying both the host genome (2) where they replicate as part of the host genome. At a frequency of approximately $10^{-2}$ to $10^{-5}$ per cell division, the prophage escapes CI repression, replicates, and lysses the host (3).

The genetic map showing the approximate organization of functional blocks and, below the map, the location of the genes up and downstream regulated as revealed in this study. The lam gene is embedded between two different tail-fiber genes. The genome length is 48.5 kb, approximately 75 genes. See [2,29].

### Results

#### Expression Profiles

Our arrays were designed to assay the expression levels of 4,589 E. coli and 73 λ open reading frames during exponential growth in tryptone broth, standard growth conditions for experiments with λ lysogens [2]. The results are summarized in Figure 2 and Table 1, in which we restrict our analysis to changes in gene expression levels of approximately 2-fold ($\log_2$) levels of approximately one) or more.

### Host Gene Expression in λ Lysogens: The pckA Gene

The most striking change is in the expression level of pckA, the gene coding for phosphoenolpyruvate carboxykinase [12]. PckA in the presence of ATP converts oxaloacetate to phosphoenolpyruvate [13]. Mutant pckA strains grow less well on succinate and other carbon sources that feed into oxaloacetate [14], although phosphoenolpyruvate can also be synthesized from succinate via phosphoenolpyruvate synthase [13]. We expected that the down-regulation of pckA transcripts in lysogens might lead to a similar problem. Figure 3 compares the generation times of lysogens and nonlysogens of both λ and another temperate phage, λimm434, on glucose and on succinate as the sole carbon source (the λimm434 cl and operator sequences replace the λ homologs in λimm434; [15,16]). There is very little difference between lysogen and nonlysogen growth rates on glucose, but on succinate it is increased at least 30% in λ lysogens and 20% in λimm434 lysogens. These are substantial changes in fitness: The lysogen will be reduced by 90% in 17 generations.

### Table 1. Host and Viral Genes Regulated in λ Lysogens

| Gene | M | A | p-Value | Product |
|------|---|---|---------|---------|
| pckA | -3.33 | 13.41 | 3.29E-34 | Phosphoenolpyruvate carboxykinase |
| b2002 | -1.11 | 9.36 | 3.50E-10 | CP4–44 prophage; putative DNA repair protein (RuvD family) |
| nagA | -0.861 | 11.38 | 3.18E-20 | N-acetylgalactosamine-6-phosphate deacetylase |
| hdeA | -0.817 | 10.24 | 1.64E-07 | Protein hdeA precursor |
| hp | -0.798 | 10.72 | 4.60E-14 | Leucine-responsive regulatory protein |
| LMD_rexB | 0.902 | 9.09 | 2.18E-18 | Exclusion |
| LMD_ea47 | 0.845 | 7.23 | 6.05E-07 | Putative protein |
| b0574 | 0.941 | 7.5 | 1.83E-21 | CysB, possible component of the copper transport system |
| LMD_ea22 | 1.08 | 7.67 | 1.37E-09 | Putative protein |
| LMD_kit | 1.20 | 5.13 | 2.76E-11 | Host-killing |
| b0572 | 1.34 | 7.12 | 1.49E-17 | CysC, putative outer membrane protein involved in copper transport |
| b0557 | 1.43 | 9.69 | 1.82E-15 | DL12 prophage; bacteriophage λ Bor lipoprotein homolog |
| LMD_nin221 | 1.47 | 5.76 | 3.34E-13 | Phosphoprotein phosphatase |
| LMD_bor_L | 1.56 | 9.36 | 2.23E-14 | Bor protein precursor |
| LMD_orf-B | 1.63 | 6.02 | 1.75E-16 | Hypothetical protein |
| LMD_lam_L | 1.88 | 9.48 | 3.83E-19 | Outer host membrane |
| LMD_int | 2.03 | 7.46 | 1.06E-25 | Integration protein |
| LMD_rexB | 2.57 | 9.55 | 9.61E-21 | Exclusion |
| LMD_cl | 3.04 | 7.93 | 4.68E-21 | Repressor |
| LMD_E | 3.16 | 7.83 | 1.17E-14 | Capsid component |
| LMD_rexA | 3.74 | 8.18 | 4.07E-27 | Exclusion |
| LMD_cl_L | 5.2 | 9.04 | 3.45E-33 | Repressor |
| LMD_rexA | 6.57 | 8.83 | 3.94E-32 | Exclusion |

**Genes and genes prefixed with "LMD."**

**M** is log (of the expression level ratio of lysogen/nonlysogen (see also Figure 2, caption).

**A** is log (channel 1/channel 2)**,$^a$** a measure of the brightness of the fluorescent signal.

**p-Values** based on the pooled data taken from 48 individual measurements using 12 different samples taken from exponentially growing cells.

**Product** function based on [19] and GenBank [gi:9626243]. The C. elegans primer was designed to amplify 4,319 open reading frames and is therefore very heterogeneous in target length. The λ set of 73 primer pairs contained some duplicate targets, for example LMD_rexB, LMD_int, LMD_rexB, where the last set was approximately 500 nucleotides long and the other set contained approximately 70 nucleotides.

DOI: 10.1371/journal.pbio.0030229.g001

---

**Notes:**

- **A** is log (channel 1/channel 2), a measure of the brightness of the fluorescent signal.
- **p-Values** based on the pooled data taken from 48 individual measurements using 12 different samples taken from exponentially growing cells.
- **Protein function** based on [19] and GenBank [gi:9626243]. The C. elegans primer was designed to amplify 4,319 open reading frames and is therefore very heterogeneous in target length. The λ set of 73 primer pairs contained some duplicate targets, for example LMD_rexB, LMD_int, LMD_rexB, where the last set was approximately 500 nucleotides long and the other set contained approximately 70 nucleotides.

---

**DOI:** 10.1371/journal.pbio.0030229.g001
homology is with a 16/17-base pair (bp) match to OL2, and map to the 370-bp sequence upstream of it occurs within the open reading frame ofatorial sequences with a 15/17-bp match, two to OR1 and two for an inner membrane lipoprotein. There are four addi-
duplicate. Thus each time point is the average of four datasets, and the data in Table 1 and Figure 2 represent 48 arrays probed with cDNA from microarrays in duplicate. For each time point, Cy3 and Cy5 labels were reversed, and reversed labels were also used to probe the microarrays in duplicate. Thus each time point is the average of four datasets, and the data in Table 1 and Figure 2 represent 48 arrays probed with cDNA from exponentially growing cells. The dataset on which Table 1 is based may be found in supplemental Table S1.

doubling time when these strains are grown on succinate as
doubling time differs by 20%.

The strongest
5% in 34 generations, and 99.9% in 50 generations when the
doubling time differs by 20%.

The E. coli genome there are many sequences that exhibit
homology to the λ operator [17,18]. The highest
homology is with a 16/17-base pair (bp) match to Oγ2, and
it occurs within the open reading frame of rlpB, a gene coding
for an inner membrane lipoprotein. There are four additional
sequences with a 15/17-bp match, two to O1γ1 and two to O1γ2. They are in the coding regions of rlpB and jbcG for
O1γ1 homologs, and hmbA and yigQ for O1γ2 homologs, none
of whose expression pattern is altered by our criteria. By
relaxing our search for homologies to 11-mers, we find 244
more sites, but in all of these cases there are gaps in the
matching strings. Some of these map in possible regulatory
regions of the genome. The strongest λ homologies, however,
map to the 370-bp sequence upstream of pekA (Figure 4).

There are homologies to other temperate phage operators
in this region as well. Phages H-19B, 434, 21, and P22 are four
such examples. The presence of these sequences suggests that
the phenotypes of the two lysogens summarized in Figure 3
might be caused by cl binding to these related operator sites.

We first examined this idea by comparing the growth rates
of strains carrying a cl-expressing plasmid to the parental
strain carrying an empty plasmid (see Figure 3, DH5zPro(pcl))
versus DH5zPro(pcl3)). There is a dramatic increase in
doubling time when these strains are grown on succinate as
the only carbon source (t = 139 min vs. 278 min), and very
little difference on glucose.

These results support the idea that cl expression depresses
growth rate, but the data do not distinguish between direct
and indirect effects. To make this distinction, we used real-
time PCR to measure lacZ message in a strain where lacZ was
fused to a 370-bp pekA upstream sequence (Table 2). Four
different strains were compared: W3350 and W3350 (λ) as
baseline controls (lines 1–4) and two different strains carrying
lacZ on a plasmid (lines 5–10). One of these, DH5zPro(-
placZpekCl), does not have a promoter and serves as a control
on lacZ transcript levels. The other, DH5zPro(ppekaLacZpekCl),
has the lacZ gene fused to a 370-bp upstream pekA sequence.
Both strains also carried either cl on a plasmid, or the same
plasmid lacking the cl gene.

The chromosomal pekA transcript is down-regulated about
3-fold in lysogens compared to wild type (lines 1–4). Apparently cl acts directly on the pekA upstream sequence,
because cl expression regulates lacZ transcript levels in strains
where lacZ is fused to the pekA upstream sequence (lines 5–10).
cl message is undetectable in nonlysogens, as expected (line
2), and the pekA transcript level is down-regulated 3-fold in a
lysogen (line 1 compared to line 3). When cl is produced from
a plasmid, it depresses pekA message approximately 2-fold
(lines 5 and 8). In the pekAlacZ fusion, although the
dorf decrease in lacZ transcript level is not as large as it is in
the previous examples, it is nonetheless down-regulated 25% to
30%. Because lacZ transcript levels are quite high in strains
in which the lacZ gene lacks a promoter altogether (line 7),
these smaller changes occur on top of a high background,
possibly due to the high copy number of this plasmid. If we
correct for this high background using these data, then the
difference is more substantial, an approximately 35% reduction in transcript level in the strain carrying upstream
pekA sequences. We note that even though the change in
transcript number when lacZ is controlled by the pekA
promoter region is not large, it is reproducible, and more-
over, it is of the same order of magnitude as the changes in growth rate documented in Figure 2.

Taked together, these data suggest that cI binds to the upstream pckA sequence and down-regulates transcription. To define this interaction further, we asked if purified cI could bind to the pckA upstream sequence in a band shift assay. In these experiments, a 370-bp upstream region (Figure 4) was labeled with 32P using the polymerase chain reaction assay. This probe is authentic because it can be competed away with unlabeled upstream radioactive probe is authentic because it can be competed.

Table 2. lacZ Is Regulated by cI When It Is Fused to the pckA Promoter Region

| Number | Strain          | Transcript | mRNA Copy Number |
|--------|-----------------|------------|------------------|
| 1      | W3350           | pckA       | 1.0E+4           |
| 2      | pckA            | cl         | 5.0E+3           |
| 3      | W3350(l)        | pckA       | 1.0E+4           |
| 4      | cl              | pckA       | 1.0E+4           |
| 5      | DH5αPro(placZpck) | pckA   | 1.0E+4           |
| 6      | cl              | lacZ       | 5.0E+3           |
| 7      | lacZ            | pckA       | 5.0E+3           |
| 8      | cl              | lacZ       | 5.0E+3           |
| 9      | lacZ            | pckA       | 5.0E+3           |
| 10     | lacZ            | pckA       | 5.0E+3           |

Here we used real-time PCR to analyze the copy number of cl, lacZ, p, and pckA transcripts under different conditions. Lines 1 through 4 are the copy numbers per cell of cl and pckA transcripts in E. coli W3350 and W3350(l). Plasmid pACY177 was used as a control. LacZ is fused to the lacZ coding sequence (lines 5 through to 7), with an insertion of the pckA promoter region fused to the lacZ promoter (lines 8 through to 11), and for the experiment corresponding to line 1, IPTG was also added to the growth medium. Data is presented as the mean and standard deviation.

DOI: 10.1371/journal.pbio.0030229.t002

 marketed here is restricted to Gram negative bacteria (results not shown).

Host Gene Expression in λ Lysogens: Other Genes

In addition to pckA, seven other host genes appear to be regulated approximately 2-fold in the lysogen (see Figure 2; Table 1). bO557 is a bO homolog contained in the DLP12 prophage sequence resident in these strains [19]. Because it is 91% homologous to λ bO, this apparent increase in expression of a host gene is probably due to hybridization between λ prophage bO transcripts and the DLP12 resident prophage. We have not explored the significance of the six other transcript profiles shown here, other than to note that two up-regulated genes are copper transporters, and, like bO and bO (20), membrane proteins [19]. Finally, one of the down-regulated genes, bO2002, is also embedded in another defective prophage, CP4-44, although it has no sequence homology with λ and is therefore likely to be directly or indirectly regulated by cl.

λ Gene Expression

In general our results are consistent with the literature on λ gene expression patterns in lysogens (summarized in [2]). The promoter regulating cl expression is known to regulate the levels of cl, rexA, and rexB, as noted earlier, and our data clearly show elevated transcript levels. Likewise, both lom and bO are known to be transcribed in lysogens [9,20]. The Lom protein makes λ lysogens more resistant to guinea pig serum [9], and the Lom protein is involved in buccal cell adhesion in the gut [21]. Both are outer membrane proteins. int required for integration of the prophage into the genome, and xis, required for excision of the prophage from the genome when the prophage escapes repression, are expressed from the cl-independent promoters pl and pl and are active when the lytic/lysogenic decision is made and during early lytic multiplication [6]. Our data reveal, however, that int is also expressed in the lysogen, suggesting a possible strategy for

DOI: 10.1371/journal.pbio.0030229.g003
stabilizing the prophage by kinetic means, in that elevated levels of Int protein might help stabilize the lysogenic state by shifting the equilibrium toward integration. This possibility would be in addition to the role that cI plays by repressing the functions needed for viral replication and packaging.

The product of the $E$ gene is a procapsid protein essential for viral packaging. It is encoded in a polycistronic late message (see Figure 1), and is elevated at least 3-fold in the lysogen. $E$ is part of a polycistronic message coding for genes needed for viral assembly, and thus finding elevated levels of $E$ transcript is unexpected. However, in addition to cI repression of $\lambda$ genes, it has been noted that there are strong Rho-independent transcription terminators flanking the $E$ gene, and this has led to the idea that transcription terminators may add an extra level of prophage regulation, helping to silence gene expression by terminating aberrantly initiated polycistronic message production and translation [22]. Our finding of $E$ up-regulation is consistent with this idea. In this context, it may also be significant that a similar termination sequence lies just downstream of $\text{int}$.

Finally, our microarray analysis suggests that five other $\lambda$ open reading frames are also expressed in the lysogen: $\text{orf}-64$, $\text{nin221}$, $\text{ea22}$, $\text{kil}$, and $\text{ea47}$. Their transcripts are all elevated 2-fold or more. Whether or not there is a functional reason for this will require additional analysis.

In the microarray experiments, it may seem surprising that genes transcribed in the lysogen are not elevated many hundred- or even thousand-fold, because we are comparing ratios of samples with and without the $\lambda$ genome. One has to remember, however, that background fluorescence is always present, and thus the reading is never zero, even from blank regions of the array.

**Discussion**

Our results show that there is strong interaction between the host and parasite genome in this model system, and we can ask what role this interaction plays in the evolution of fitness in these populations.

We note first that lysogen growth rates on succinate are dramatically reduced both for $\lambda$ and $\lambda\text{imm434}$ lysogens (see Figure 3). These are very large fitness changes, in the 20% to 30% range per generation, a profound disadvantage for lysogens in a competitive environment in which succinate is the carbon source and, by extension, gluconeogenesis is important.

The second clear result is that there are at least seven DNA sequences with varying degrees of homology to the $\lambda$ operator sites upstream of the $\text{pckA}$ gene. These sites are homologous to operator sequences used by other temperate phage, and they lie either close to the $-10$ to $-35$ polymerase binding site, or between this region and the ribosomal binding site (see Figure 4). One, the OL2 homology of phage 21 may lie too far upstream to be considered part of the $\text{pckA}$ regulatory domain. Were there only one or two $\lambda$-specific sequences in this region, we might conclude that they were there by chance.
alone. However, the many distinct potential cI binding sequences for H-19B, 434, 21, and P22 make the chance hypothesis unlikely. Note that these sites are dissimilar, reflecting the different target sequences for the different phage repressors—they are not simply differences within a single short consensus sequence. Although the λ O2 sequence is at best a half operator, the others show substantial homology, and the 370-bp pckA sequence binds cI in vitro with an affinity at least as strong as the authentic λ consensus sequence (Figure 5).

The clustering of operator homologies suggests that there is strong selection pressure maintaining these sites and that they are an important aspect of lysogeny fitness, one in which the regulation of a host gene, rather than the production of a phage product, confers increased fitness. There is currently insufficient knowledge about whole-genome E. coli expression patterns in different environments to speculate about what these selective pressures might be, but we suggest that lysogens may preferentially survive because of their lower growth rates in a glucose-poor environment—which would be the case, for example, if the immune system more effectively attacks rapidly growing cells in such an environment—or it could be that an aspect of lysogeny itself must be offset by down-regulating the pckA gene. Independent of the mechanism, our main conclusion is that the multiple potential operator sites lying upstream of the pckA gene suggest strong positive selection for this subtle host–parasite interaction, one that can be added to the known advantages of λ protection from infection by T2 and T4 phage, and resistance to host serum factors.

How do these results compare to other studies with viral-infected cells? Following animal virus infection, many host genes are turned on and off. For example, in one early study of cells infected with human cytomegalovirus, 1,400 of the 12,626 genes surveyed changed by a factor of four or more following infection, and several of these changes pointed to the central role played by the immune system in a productive infection [23]. Other studies in a wide variety of infected cells confirm these results—most host genes are both up- and down-regulated—but to our knowledge these changes in gene expression have not been firmly tied to phenotypic changes in host–virus dynamics.

Materials and Methods

Strains. W3350 is from the E. coli Genetic Stock Center (CGSC #9076) (http://cgsc.biology.yale.edu), galK20Δc, galT22, lamC, and in(rndD64εndE). DH5α is from Clontech (Palo Alto, California, United States), deoR, endA1, gyrA96, hsdR17(rk +, K-), recA1, relA1, supE44, thi-1, ΔlacZΔM15, F−, F′ proAE242, lacI2, lacZΔM15, F−, F′ proAE242, and λ4Δ344 is from Dr. R. Weissberg, and λ4Δ344 is from Dr. D. Kaiser.

Microarray analysis. A total of 4,539 E. coli MG1655 open reading frames and 73 λ genes were amplified by PCR, purified by ethanol precipitation, and verified by gel electrophoresis. The E. coli MG1655 genome open reading frame primer set is from Sigma-Genosys (Woodlands, Texas, United States), and 73 primer pairs for λ genes were designed by using the Primer3 program (http://workbench.sbc.edu) and synthesized by Integrated DNA Technologies (IDT; Coralville, Iowa, United States). A list of the genes and primers is available from the corresponding author. The purified PCR products were resuspended in 50% DMSO and printed onto Corona Ultra GAPS slides (Corning, New York, United States) using OmnimGrid from Genomic Solutions (Ann Arbor, Michigan, United States). E. coli W3350 and W3350(λ) were grown in Tryptone medium with aeriation, and 12 samples from each culture were taken about every 6 min between OD₆₀₀ 0.1 and 0.6. RNA samples were extracted using the Qiagen RNaseasy kit (Valencia, California, United States). RNA labeling and microarray hybridization were as described [24]. For each time point, W3350 cDNA samples were labeled with Cy3, and W3350(λ) cDNA samples were labeled with Cy5. The samples were mixed and used to probe the microarrays in duplicate. For each time point, Cy3- and Cy5-labeling schedules were reversed, and the reverse-labeled cDNA samples were also used to probe the microarrays in duplicate. Thus each time point is the average of four datasets, and the data in Table 1 and Figure 2 represent 48 arrays probed with cDNA from exponentially growing cells. The hybridized arrays were scanned using an Axon 4000B scanner (Sunnvale, California, United States), and the data were analyzed using limmaGUI (http://bioinf.wehi.edu.au/limmaGUI) with Bioconductor packages (http://www.bioconductor.org).

Bacterial doubling times. Strains were grown in M9 minimal media [25] supplemented with 1 mM β12, and either 0.4% glucose or 0.4% succinate as the only carbon source.

Band shift assay. λ cI protein was purified using the PRO Tet 6×HN Bacterial Expression System (Clontech) as described [26]. The PCR product of the pckA upstream sequence (pPckA), which is the 370-bp E. coli genomic region 3530644 to 3530833 covering the pckA promoter region, was amplified using the primers TGGTTATCGGAAATCAAAGGTG and GCGCTTGAGCCAATAATGGTACG, and labeled with 32P-PCR Primer (Pierce). PCR-labeled DNA were mixed in SaeI buffer [27] with or without competitors (pPckA, λ O2, or randomized λ O2 DNA), incubated on ice for 2 h, and run on a precast 10% TBE native polyacrylamide gel from Bio-Rad (Hercules, California, United States). Gels were dried and exposed to x-ray films. The competitor λ O2 was amplified by PCR using primers GCGCTTGAGCCAATAATGGTACG and GCCGATGCGTAAATCTTCA, and is 184 bp long. Another competitor, randomized λ O2, was synthesized by IDT and is 17 bp long.

Real-time PCR analysis. The λ cI expression vector pE133-cI (‘‘pel’’ in the text) was constructed by cloning the cI gene (coordinate positions 37227 to 375490 on the λ genome) into the expression vector pPROTeinE133 (Clontech). The E. coli pckA upstream sequence fused to the lacZa coding sequence, the lacZa promoter sequence followed by the lacZa coding sequence, or the lacZa coding sequence alone were cloned into the λ cI sites of pACYC177. The pE133-cI vector and each of the three modified pACYC177 vectors were electroporated together into DH5α Pro electro-competent cells (Clontech). The transformed strains were grown under different conditions, depending on the experiment. RNA samples were extracted using Qiagen RNaseasy kit (Valencia, California, United States). 16S ribosomal RNA was used as a reference to normalize message copy numbers, assuming 20,000 copies of the rRNA molecule per cell (Table S1). Primers used were 5′-CCACGGCTCTCCTCGTATACG-3′ and 5′-GGGGCTTCTGGCGTTATACG-3′ were used to detect lacZa, primers ATGGCGGTTAACAAGTTTTGA and TAGTTAACCCCGCGCTCAT were used to detect pckA, and primers GTGGAAGTATCTTCCATGAC and ACTAGGATATCCTCACCAC were used to detect cI.

Supporting Information

Table S1. Host and Viral Genes Regulated in λ Lysogens

Columns A, B, and C are the spot coordinates; Column E: The λ gene, Column D: the number of ORFs from the original ORF primer set for the λ gene; and Column F: the expression level ratio of lysogen to nonlysogen (see also Figure 2 caption); Column H: A = log (channel 1) (channel 2)^2, a measure of the brightness of the fluorescent signal; Columns I, J, and K: Statistical analysis of the pooled data from 48 individual measurements using 12 different samples taken from exponentially growing cells. The E. coli MG1655 primer set from Sigma-Genosys (Woodlands, Texas, United States) was designed to amplify 4,539 open reading frames and is therefore very heterogeneous in target length. The λ set of 73 primer pairs contained some duplicate targets, for example, LMD_3039 and LMD_3041; in which the L set was approximately 100 nucleotides long and the other set contained approximately 70 nucleotides. The LMD primer set is available from the corresponding author (ecox@princeton.edu).

Found at DOI: 10.1371/journal.pbio.0030229.s001 (764 KB XLS).
Accession Numbers

The GenBank (http://www.ncbi.nlm.nih.gov/Genbank) accession numbers for the genes and gene products discussed in this paper are b0557 (GeneID 948980), b2002 (GeneID 946514), cij (GeneID 1238683), E (GeneID 2703482), eae22 (GeneID 2703469), eae47 (GeneID 2703536), hmnpA (GeneID 947018), int (GeneID 2703470), kil (GeneID 2703471), nes221 (GeneID 2703476), ofv-64 (GeneID 2703478), pbc (GeneID 946647), pekA (GeneID 945667), rexl (GeneID 2703537), relB (GeneID 2703517), regB (GeneID 948980), regC (GeneID 1037318), and kil genes (NC 001146). The GenBank accession number for the E. coli MG1655 primer set is U00096.

References

1. Ackermann H-W, DuBow MS (1987) Viruses of prokaryotes. Boca Raton (Florida): CRC Press. 680 p.
2. Hendrix RW (1985) Lambda II. Cold Spring Harbor (New York): Cold Spring Harbor Laboratory Press. 694 p.
3. Adams MH (1959) Bacteriophages. New York: Interscience Publishers. 592 p.
4. Wagner PL, Waldor MK (2002) Bacteriophage control of bacterial virulence. Infect Immun 70: 3985–3993.
5. Hendrix RW, Smith MC, Burns RN, Ford ME, Hatfull GF (1999) Evolutionary relationships among diverse bacteriophages and prophages: All the world’s a phage. Proc Natl Acad Sci U S A 96: 2192–2197.
6. Ptashne M (1992) A genetic switch: Phage lambda and higher organisms. Cambridge (Massachusetts): Cell Press, Blackwell Scientific Publications. 192 p.
7. Edlin G, Lin L, Kudrnx R (1975) Lambda lysogens of E. coli reproduce more rapidly than non-lysogens. Nature 255: 735–737.
8. Dykhuizen D, Campbell JH, Rolfe BG (1978) The influences of a lambda prophage on the growth rate of Escherichia coli. Microbios 23: 99–113.
9. Barondess JJ, Beckwith J (1995) bor gene of phage lambda, involved in serum resistance, encodes a widely conserved outer membrane lipoprotein. J Bacteriol 177: 1247–1253.
10. Barondess JJ, Beckwith J (1990) A bacterial virulence determinant encoded by lysogenic coliphage lambda. Nature 346: 871–874.
11. Spanier JG, Cleary PP (1980) Bacteriophage control of antiphagocytic interests exist. J Exp Med 152: 1393–1406.
12. Medina V, Pontarollo R, Glaeske D, Tabel H, Goldie H (1990) Sequence of Escherichia coli K-12 adhesion to human buccal epithelial cells. FEMS Microbiol Lett 156: 129–132.
13. Juhala RJ, Ford ME, Duda RL, Youton A, Hatfull GF, et al. (2000) Genomic sequences of bacteriophages HK017 and HK022: Pervasive genetic mosaicism in the lambdoid bacteriophages. J Mol Biol 299: 27–51.
14. Browne EP, Wing R, Coleman D, Shenk T (2001) Altered cellular mRNA levels in human cytomegalovirus-infected fibroblasts: Viral block to the accumulation of antiviral mRNAs. J Virol 75: 12191–12196.
15. Hegde P, Qi R, Abernathy K, Gay C, Dharap S, et al. (2000) A concise guide to cDNA microarray analysis. Biotechniques 29: 548–550, 552–554, 556.
16. Sambrook J, Russell DW (2001) Molecular cloning: A laboratory manual. Cold Spring Harbor (New York): Cold Spring Harbor Laboratory Press. 999 p.
17. Samiuer KT, Foquet M, Guo L, Cox EC, Craighead HG (2005) Lambda-repressor oligomerization kinetics at high concentrations using fluorescence correlation spectroscopy in zero-mode waveguides. Biophys J 88: 2145–2153.
18. Neidhart FC, Ingraham JL, Schaechter M (1990) Physiology of the bacterial cell. A molecular approach. Sunderland (Massachusetts): Sinauer Associates. 506 p.
19. Sanger F, Coulson AR, Hoefn GH, Hill DF, Petersen GB (1952) Nucleotide sequence of bacteriophage lambda DNA. J Mol Biol 16: 729–773.
20. Smyth GK (2004) Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat Appl Genetics Mol Biol 3: 3.
21. Neely MN, Friedman DI (1998) Arrangement and functional identification of genes in the regulatory region of lambdoid phage H-19B, a carrier of a Shiga-like toxin. Gene 225: 105–113.
22. Potete AR, Ptashne M, Ballivet M, Eisen H (1980) Operator sequences of bacteriophages P22 and 21. J Mol Biol 157: 81–91.