Commitment to Lysogeny Is Preceded by a Prolonged Period of Sensitivity to the Late Lytic Regulator Q in Bacteriophage λ

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A key event in development is the irreversible commitment to a particular cell fate, which may be concurrent with or delayed with respect to the initial cell fate decision. In this work, we use the paradigmatic bacteriophage λ lysis-lysogeny decision circuit to study the timing of commitment. The lysis-lysogeny decision is made based on the expression trajectory of CI. The chosen developmental strategy is manifested by repression of the pr and pl promoters by CI (lysogeny) or by antitermination of late gene expression by Q (lysis). We found that expression of Q in trans from a plasmid at the time of infection resulted in a uniform lytic decision. Furthermore, expression of Q up to 50 min after infection results in lysis of the majority of cells which initially chose lysogenic development. In contrast, expression of Q in cells containing a single chromosomal prophage had no effect on cell growth, indicating commitment to lysogeny. Notably, if the prophage was present in 10 plasmid-borne copies, Q expression resulted in lytic development, suggesting that the cellular phage chromosome number is the critical determinant of the timing of lysogenic commitment. Based on our results, we conclude that (i) the lysogenic decision made by the CI-Cro switch soon after infection can be overruled by ectopic Q expression at least for a time equivalent to one phage life cycle, (ii) the presence of multiple λ chromosomes is a prerequisite for a successful Q-mediated switch from lysogenic to lytic development, and (iii) phage chromosomes within the same cell can reach different decisions.

Materials and Methods

Strain and plasmids construction. The oligonucleotides used in the construction of strains and plasmids are shown in Table 1. In the construction of plasmid pBADQ, the Q-coding region from bacteriophage λ was amplified by PCR using primers QUP and QDN. The resulting DNA fragment was digested with NheI and PstI and inserted into plasmid pBAD24 (14) using the same sites. The sequence of the cloned region was verified. Plasmid pSEM3058 was constructed by inserting the attB site, fused to a DNA sequence encoding the P5 variant of the fast-folding GFPmut2 protein (15), into plasmid pLG338 (16). Escherichia coli MG1655 cells carrying plasmid pSEM3058 were infected with wild-type λ (λWT). Integration of the λ chromosome into pSEM3058 but not into the chromosome was verified by PCR in the selected clones. To create plasmid pSEM3141B, we first ligated the EcoRV-PstI fragment of pEM7/Zeo (Invitrogen), which assembly, DNA packaging, and lysis are transcribed from the pr promoter and require Q for their expression (10, 11). Q and CII are transcribed from the same polycistronic mRNA, but Q activity is significantly delayed compared to CII activity (12, 13). This delay has been suggested to ensure sufficient time for completion of the decision-making process before Q activates expression of the late lytic genes (12). It is, however, not known whether sufficient CII accumulation represents a point of irreversible commitment to lysogeny or whether the decision remains reversible during the progression to the stably inherited prophage state.

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Effect of Q Expression on Lysogeny in \( \lambda \)

FIG 1 Arrangement of the key regulatory elements on the bacteriophage \( \lambda \) chromosome. The \( \mathbf{qR} \) transcript encodes the transcription-regulatory proteins Cro and CI, the Q antiterminator protein, and the replication proteins O and P. Transcription of the late genes is initiated at the \( \mathbf{pR} \) promoter and require the presence of the Q protein to proceed through the \( \mathbf{R}^+ \) terminator. The late genes encode proteins required for phage capsid production, DNA packaging, and host cell lysis.

contained the zeocin resistance cassette, with the PstI-PvuII fragment of pMOD-3( R6K \( \mathbf{cry} \)/MCS), which contained the R6K \( \mathbf{cry} \) replication origin. The plasmid was maintained in Pir1 cells (Invitrogen), which supplied the replication protein \( \mathbf{r} \). To add the attB site, the resulting plasmid was PCR amplified using the ATTBR6 and ZEOR1 primers, and the PCR product was digested with EcoRI. The DNA fragment obtained was circularized by T4 DNA ligase to generate pSEM3141B. Pir1 cells which carried the pSEM3141B plasmid were infected by K. \n
TABLE 1 Oligonucleotides used in the construction of strains and plasmids

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<thead>
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<th>Name</th>
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<tr>
<td>ATTBR1GFP</td>
<td>TTTTGAATTTCCTGCTTATTTTATACACTAAGTCACTGCGATATTAAGAGGAAGATACATATCGCC</td>
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<tr>
<td>GFDPNBGLII</td>
<td>TTTTTATGATACTTTATATTGTAAGCCTCATCCATGCCC</td>
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<td>CYSG RT R</td>
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Effects of Q Expression on Expression of Late Genes from a Promoter Located between Genes P and Q

- **Results**: Premature Q expression promotes lytic development. A previous study showed that constitutive expression of the late genes from a promoter located between genes P and Q in the \( \lambda qin101 \) mutant gives clear plaques, indicating failure to lysogenize (19). However, the \( \lambda qin101 \) Q mutant gave small plaques but lysogenized normally (19). These observations suggested that expression of Q makes the decision independent of the CI-controlled expression trajectory. To test this hypothesis we constructed a plasmid which allowed arabinose-controlled expression of the Q protein

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In the absence of arabinose, infection of pBADQ-containing E. coli MG1655 cells by wild-type \(\lambda^{WT}\) phage resulted in turbid plaques. However, in the presence of arabinose, the plaques obtained were uniformly clear (Fig. 2). Thus, premature expression of Q indeed appears to overrule CII-mediated decision-making. We note that the ability of the arabinose-induced cells to form plaques proves that functional phage progeny is produced from cells that contain Q at the time of infection.

A stably inherited prophage is insensitive to Q expression. Interestingly, expression of the late genes in a single-copy \(\lambda\) lysogen had no visible effect on colony growth. To confirm that cell growth is indeed unaffected by Q expression, we compared the growth of \(\lambda\) lysogens to that of the parental MG1655 cells in liquid medium, in both the presence and absence of Q expression (Fig. 2A, squares). Induction of Q expression in E. coli MG1655 by 0.4% arabinose had no visible effect on growth, even if the strain contained a single-copy \(\lambda\) prophage.

Induction of Q expression postinfection can alter the lysis-lysogeny decision. As shown in Fig. 2, the Q protein was able to direct the decision toward lytic development when present in the cells at the time of infection. However, in cells carrying a stably inherited prophage, expression of Q had no effect (Fig. 3). Thus, at some point between the time of infection and the stably inherited prophage state, the infected cell commits to lysogeny and becomes insensitive to expression of Q and the late lytic genes.

To get insight into the timing of the decision and commitment process, we tested how long Q expression can affect the lysis-lysogeny decision after infection. Cells carrying the pBADQ plasmid were mixed with \(\lambda^{WT}\) phage at an average phage input (API) of 6 (to favor lysogeny). To synchronize the cells with regard to infection time, the mixture was incubated at 20°C to allow adsorption of the phage particles to the host cells (see Materials and Methods) before it was moved to 37°C to allow injection of phage DNA into cells. Furthermore, any remaining free phage particles were inactivated after 5 min at 37°C by the addition of a 100-fold surplus of heat-killed, maltose-induced MG1655 E. coli cells in sodium citrate to prevent further infection of the live cells. At different times postinfection, an aliquot of the synchronized culture was transferred to a culture tube containing arabinose to induce Q expression. The fractions of cells that chose the lysogenic pathway in the

![FIG 2](image)

**FIG 2** Growth of wild-type \(\lambda\) on host cells expressing the Q protein. Top agar containing 0.4% \(\alpha\)-arabinose was mixed with E. coli MG1655 cells carrying the pBADQ plasmids preadsorbed with wild-type \(\lambda\) (\(\lambda^{WT}\)) phage and poured on the surfaces of TB plates containing 100 \(\mu\)g/ml ampicillin and 0.4% arabinose. Clear plaques were formed (right panel), indicating that the lytic pathway was chosen. In the absence of arabinose (left panel) turbid plaques were formed.

![FIG 3](image)

**FIG 3** Effect of Q expression on cell growth and phage production. (A) Cells were grown overnight and diluted in fresh LB medium (containing 50 \(\mu\)g/ml ampicillin and 30 \(\mu\)g/ml kanamycin) at the zero time point. At 65 min, \(\alpha\)-arabinose was added at 0.4% concentration (solid symbols). Control cultures were grown without \(\alpha\)-arabinose (empty symbols). The optical densities of the cultures were recorded at different times. Triangles and dashed lines represent E. coli MG1655 cells carrying plasmids pBADQ and pSEM3058. Squares represent cells carrying a \(\lambda\) prophage (integrated into the chromosomal attachment site) and plasmids pBADQ and pLG338. Circles represent cells carrying plasmids pBADQ and pSEM3058a. pSEM3058a carries a single \(\lambda\) prophage and is present at about 10 copies per cell. (B) Cells were grown and induced as for panel A but using a range of arabinose concentrations. After 23 h of growth, optical densities of the cultures were recorded (circles, right axis), and the phage content of the lysates was tested by plaque assays (squares, left axis).
arabinose-induced subcultures as well as the original, uninduced culture were recorded by plating for colonies at 60 min postinfection (see the schematic of the experiment in Fig. 4). Subsequently, surviving cells which had become lysogenic for \(\lambda\) were distinguished from those which had escaped infection entirely by testing the colonies for immunity to \(\lambda\).

Figure 4 shows the fraction of lysogens in the arabinose-induced subcultures relative to the number of lysogens in the original culture, which did not contain arabinose. It is evident that expression of Q in trans shortly after infection resulted in a reduction of the lysogenic cell fraction by more than 90%, in agreement with the plaque phenotype shown in Fig. 2. Interestingly, we found that most cells remained sensitive to Q expression long after the initial lysis-lysogeny decision had occurred. Specifically, induction of Q expression resulted in cell death even at 50 min postinfection (corresponding to a full lytic life cycle for \(\lambda\)) in more than half of the cells which otherwise would have continued the path to lysogeny (Fig. 4).

**Q expression in cells carrying 10 stably inherited prophages results in lysis.** Expression of Q in trans resulted in very different outcomes depending on whether it occurred at 50 min postinfection (the last time point in Fig. 4) or many generations postinfection (single-copy lysogen [Fig. 3A]). One important difference between lysogenic cells in these two situations is that a freshly infected cell carries multiple phage \(\lambda\) genomes per host chromosome, because the phage genome is initially replicated every 2 to 3 min following infection, regardless of whether the outcome of infection will be lysis or lysogeny (20). In contrast, the established lysogen carries only one \(\lambda\) genome per host chromosome. To test whether the number of \(\lambda\) genomes accounts for the insensitivity of the established single-copy lysogen to Q induction, we constructed a strain which contains about 10 plasmid-borne copies of the \(\lambda\) prophage [MG1655(pSEM3058)], the 10:1 phage gene dosage relative to that of host genes was confirmed by quantitative real-time PCR (see Materials and Methods).

The result shown in Fig. 3 (circles) demonstrates that, unlike with the single-copy prophage, the growth of the strain carrying 10 copies of the \(\lambda\) prophage was completely inhibited after induction of Q expression, and later cell lysis was observed. Induction of the pBAD promoter by arabinose is all or none; that is, subsaturating concentrations of arabinose result in a mixed population of cells with fully induced Q transcription and cells with uninduced levels of Q (21). As expected, growth inhibition depended on the concentration of arabinose in the medium and occurred in the dynamic range of the pBAD system (14) (Fig. 3B, circles). Thus, the number of phage chromosomes is a critical determinant of the effect of Q expression.

**FIG 4** Effect of the timing of Q expression on the lysis-lysogeny decision. *E. coli* MG1655 cells carrying pBADQ were infected by wild-type \(\lambda\) at an API of 6. Arabinose (0.4%) was added at the indicated time points, and after 60 min postinfection, the mixture was plated to allow colony formation by the surviving cells. Bars indicate the fraction of cells which maintained the lysogenic decision upon Q expression (number of \(\lambda\)-immune colonies from samples where arabinose was added at the indicated time postinfection divided by the number of \(\lambda\)-immune colonies from samples without arabinose addition). Error bars represent one standard deviation from the mean based on data from three independent cultures.
Q induction results in the production of infectious phage particles. The above observations raise the important question of whether late gene induction by Q results in the production of infectious phage particles, that is, true lytic development, or simply cause host cell killing without phage production. Testing the cell-free lysates by plaque assay showed that the number of infectious phage particles, that is, true lytic development, or similar to the single lysogen. To test this possibility, we used, we integrated the attB site of pSEM3058 and pBADQ indicated the presence of both attP and the plasmid-borne attB sites, besides the presence of the attL and attR sites of pSEM3058A (data not shown), suggesting the presence of both the empty plasmid and the lac-bearing plasmid in the cell population.

To understand whether these results were specific to the plasmid we used, we integrated the λ chromosome into another attB-containing plasmid. This plasmid (pSEM3141B) did not exceed the size restriction for DNA packaging. Integration of a single λ chromosome into the plasmid was confirmed by gel electrophoresis of restriction fragments (Fig. 6, lane 1). This analysis indicated the presence of the empty plasmid along with the plasmid carrying the λ chromosome even after multiple passages of the lysogen and without induction of Q. Bands specific to attP or to the linear form of the λ chromosome packed into the phage heads (cos to cos) were not observed. These results suggest that the prophage is excised spontaneously at a relatively low rate but the excised prophages remain inactive (presumably repressed by CI) in the absence of Q expression.

Induction of Q expression in cells carrying pSEM3141B resulted in growth inhibition and lysis, similar to the case with pSEM3058A. To determine whether the zeocin resistance marker from the plasmid could be transduced by the phage particles, Pir1 cells were infected with the lysate obtained after Q induction or the control lysate form uninuced cells. Transductants that had received the zeocin marker of the plasmid occurred at a low frequency from the lysate of the induced cells but not from that of the uninuced cells. These transductant colonies carried both pSEM3141B and the empty plasmid (Fig. 6, lane 2), confirming the spontaneous excision of the λ chromosome from the plasmid. Thus, we conclude that expression of Q permits the production of plaque-forming phage particles from both chromosomal and extrachromosomal DNA templates.

**DISCUSSION**

The choice between alternative cell fates is fundamental to biology. Many principles of cellular decision-making were first demonstrated in bacteriophage λ, one of the most comprehensively studied genetic systems (6). The λ genome encodes two developmental gene expression cascades, but the organization of the reg...
Critical to this hypothesis is the determination of whether or not infectious phage particles are actually produced from these dual-decision cells. Unfortunately, the experimental setup shown in Fig. 4 does not allow for quantification of released phage particles, both because of the measures taken to inactivate free phage particles in order to synchronize the initial infection (see Materials and Methods) and because the majority of cells in the culture choose the lytic pathway at the time of the initial decision, thereby masking any additional phage release from the minority of cells that initially followed the lysogenic path and later switched to lysis.

We note that the low frequency of lysogeny in our experiment (1 to 3%), is most likely due to the presence of a CII binding site on the pBADQ plasmid (at the pAQ promoter), resulting in the titration of cellular CII levels at the time of the initial decision. Nevertheless, our results show that infectious phage particles can be produced as a result of ectopic Q expression in established (multicopy) lysogenic cells. Given that the dual-decision cells observed in Fig. 4 would also contain multiple extrachromosomal λ genomes, they most likely release infectious phage particles as well. Because expression of Q has no visible effect on single lysogens but induces lysis in cells carrying multiple λ prophages, we conclude that the presence of multiple phage chromosomes is in fact a prerequisite for lytic development. In this study, Q was provided externally from a multicopy plasmid. In the natural system Q is translated from the pR mRNA, the same mRNA that encodes CII. The pR mRNA is produced at a rate of 0.17/s (25) and has a half-life of about 13 min (26), suggesting that it becomes highly abundant after infection and decays slowly after pR transcription is repressed by Cro (in the case of lytic development) or CI (in the case of lysogenic development). CII levels are expected to depend strongly on the presence of pR mRNA because the CII protein is short-lived and actively degraded by host proteases (reviewed in reference 6). Based on the results presented here, we hypothesize that to establish stable lysogeny, CII must accumulate sufficiently to suppress Q production (or activity) until the number of λ chromosomes fall below a critical level required for lytic development. The period of availability of a critical number of phage λ chromosomes thus determines the duration of the noncommitted interval in which the lysogenic course can be turned to lytic development.

The dependence of lytic development on gene dosage may serve to increase the stability of established lysogens by providing

FIG 6 Restriction analysis of plasmid pSEM3141BA. Plasmid DNA obtained from Pir1 cells carrying pSEM3141BA and pBADQ (lanes 1 and 4) and from zeocin-resistant transductants (lanes 2, 3, 5, and 6) was digested with EcoRI (lanes 1 to 3) or with NcoI (lanes 4 to 6) and separated by electrophoresis using an 0.8% SeaKem GTG agarose gel (Cambrex). The GeneRuler DNA ladder mix (Fermentas) was used as a marker (lane M). The DNA fragments labeled attL and attR have the expected size of the junctions between plasmid DNA and the λ chromosome, indicating integration of the λ chromosome into the plasmid. The EcoRI and NcoI fragments containing the attP fragment of the λ chromosome are 5,643 and 3,967 bp long, respectively, and could not be observed on the gel. The band corresponding to the empty plasmid (804 bp) is marked by an asterisk, while arrows indicate bands corresponding to the uncleaved pBADQ plasmid, which does not contain any EcoRI sites.
protection against consequences of accidental transcription of the q gene or the late genes. Notably, the stability of a double lysogen is significantly reduced compared to that of a single lysogen in the Shiga toxin-encoding lambdoid phage φ24B of E. coli O157 (27).

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REFERENCES