Establishing Lysogenic Transcription in the Temperate Coliphage 186

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A single-copy chromosomal reporter system was used to measure the intrinsic strengths and interactions between the three promoters involved in the establishment of lysogeny by coliphage 186. The maintenance lysogenic promoter p_L for the immunity repressor gene cI is intrinsically ~ 20 -fold weaker than the lytic promoter p_R . These promoters are arranged face-to-face, and transcription from p_L is further weakened some 14-fold by the activity of p_R . Efficient establishment of lysogeny requires the p_E promoter, which lies upstream of p_L and is activated by the phage CII protein to a level comparable to that of p_R . Transcription of p_E is less sensitive to converging p_R transcription and raises cI transcription at least 55-fold. The p_E promoter does not occlude p_L but inhibits lytic transcription by 50%. This interference is not due to bound CII preventing elongation of the lytic transcript. The p_E RNA is antisense to the anti-immune repressor gene apl , but any role of this in the establishment of lysogeny appears to be minimal.

Temperate bacteriophages provide model systems for studying developmental switches. One question of interest is how these phages achieve efficient and conditional transitions between the lytic and lysogenic alternative developmental states. Bacteriophage lambda, in response to DNA-damaging agents, leaves the lysogenic state and enters lytic development, a process termed prophage induction (7). The reverse transition, establishment of lysogeny, is conditional in lambda upon the activity of a lytic operon gene, cII (9), that responds to the physiology of the infected cell (24). Bacteriophage P2 is the prototype of the second major group of temperate bacteriophages of *Escherichia coli* (2, 3), and its genome shares very little DNA homology with lambda (4). P2 forms a noninducible prophage and does not require a cII -like gene in order to establish lysogeny (4). However, these traits are not shared by all P2-like phages. Bacteriophage 186 is highly related to P2 (4) yet is like lambda in being SOS inducible (28) and requiring a cII gene for efficient lysogenization (11).

Lytic and lysogenic development are controlled in these phages by a stable switch formed by the lytic and lysogenic promoters and the first genes of the divergent lytic and lysogenic operons (Fig. 1). In lysogeny, the lysogenic or maintenance promoter ($p_{RM}/p_L/p_C$) is active, resulting in production of the immunity repressor (CI/C), which represses the lytic promoter (p_R/p_C) and stimulates the lysogenic promoter. Prophage induction in lambda and 186 involves inactivation of the CI protein by activated RecA coprotease (17) or by a phage-encoded protein, Tum (10). In lytic development, the lytic promoter is active and the first gene of the lytic transcript encodes a repressor (Cro/Apl/Cox) that modulates the activity of the switch promoters (12, 15, 19). Initially, upon lytic infection of a sensitive cell, no immunity repressor is present. Establishment of lysogeny requires that adequate immunity re-

pressor is made sufficiently quickly to block lytic development. Once present, the immunity repressor stimulates the lysogenic promoter and maintains its own production. In lambda and 186, but not P2, establishment of lysogeny requires an alternative promoter for cI (p_{RE}/p_E) that lies distal to the cro and apl genes, respectively (Fig. 1). This establishment promoter is activated by the binding of CII (13, 23). The establishment transcript extends antisense across the cro/apl gene, traverses the switch promoters and the binding sites for the switch proteins, and enters the cI gene.

The activity of lambda p_{RE} appears to promote lysogeny in a number of ways. The p_{RE} promoter is much stronger than p_{RM} (16), and production of CI from the p_{RE} RNA is more efficient than from the p_{RM} transcript, which lacks typical ribosome binding sequences (21). It has also been shown that converging transcription from p_{RE} interferes with lytic transcription from p_R (20), and it has been suggested that the p_{RE} RNA might inhibit Cro production by an antisense mechanism (26), although no experiments have tested the latter hypothesis. In this study, we examined the roles of CII and p_E in the establishment of lysogeny in 186. We did not expect differences in the efficiency of CI production from the p_L and p_E transcripts, since they share the 70 bases upstream of the cI start codon; however, we wished to know to what degree CII was able to increase transcription of the cI gene. In addition, we investigated the effect of the apl gene on cI transcription from p_L and p_E and examined the interactions between these promoters and the p_R promoter.

CII increases cI transcription in the face of lytic transcription. Given the probability that the lysogenic and establishment transcripts of cI are equally well translated, the major reason expected for the involvement of p_E in the establishment of lysogeny in 186 was the enhanced transcription of cI in the face of converging transcription from the lytic promoter, which has previously been shown to reduce the activity of p_L (5). In order to compare the strengths of these promoters, $lacZ$ fusions were constructed and single copies of each were inserted into the bacterial chromosome, using the system of Simons et al. (25). To avoid the potential influence of sequence context on the promoter assay, all the comparative studies involved the

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FIG. 1. Lysis-lysogeny switches of λ , 186, and P2. CI and C are immunity repressors, and Cro, Apl, and Cox are anti-immunity repressors. The diagrams are not to scale, but the relative distances between the repressor and anti-immunity repressor open reading frames for the three switches are accurate. Note the back-to-back arrangement of the lytic (p_R) and lysogenic (p_{RM}) promoters in λ , their face-to-face arrangement in 186 (p_R and p_L) and P2 (p_e and p_c), and the different locations of the binding sites for the immunity (rectangles) and anti-immunity (filled circles) repressors. p_{RE} and p_E are the CII-activated establishment promoters of λ and 186, respectively.

same restriction fragment, carrying the three promoters under study but bearing mutations inactivating different promoters as appropriate. To inactivate the lytic promoter p_R , the -35 sequence was altered from TTTACT to CTCGAG (15), whereas to inactivate the lysogenic promoter, p_L the -10 sequence was altered from TAGATT to GCGCTT. The absence of active CII essentially inactivated the establishment promoter. The β -galactosidase activities obtained represent transcription across the control region and approximately the first 500 bp of the immunity repressor gene.

In the presence of CII, leftward transcription of cI (λ PN78) increased 86-fold, from 6 to 517 U, while converging transcription from p_R (λ PN117) was reduced 1.7-fold, from 1,278 to 760 U (Fig. 2). This effect of CII represented a 145-fold shift in the relative strengths of cI transcription and lytic transcription.

Apl has little or no effect on the establishment of transcription of the immunity repressor gene. The Apl protein binds to a series of seven direct repeats that overlap the transcription

start points of the lytic and lysogenic transcripts (6)(Fig. 1). Initial studies suggested an inhibitory role on transcription from p_L for Apl (anti- p_L)(5). Subsequently, Reed et al. (15) suggested that p_R activity was more sensitive than p_L to Apl and that the absence of Apl had little impact on the frequency with which infections followed the lytic or lysogenic pathway of infection (6). In the present study, we showed (Fig. 2) that Apl expressed from the single-copy reporter construct was sufficient to repress the lytic promoter twofold, from 2,275 U (λ PN332) to 1,278 U (λ PN117), and that Apl appeared to act in concert with CII in reducing overall lytic transcription to 760 U (λ PN117). However, despite this reduction in converging transcription, the presence of Apl made little improvement in leftward transcription, which increased from 444 U (λ PN157) to 517 U (λ PN78), which is within the 90% confidence limits; Apl also presented no barrier to the passage of RNA polymerase from p_E . These facts are in accordance with the demonstration by Dodd et al. (6) that Apl had no role in the lysis-lysogeny decision.

Promoter interactions. (i) p_E and p_L activities are additive. The strength of p_L alone measured 115 U (λ PN538) and the base changes made at p_L reduced this activity to 2 U (λ PN610), essentially inactivating it (Fig. 2). The strength of p_E in the absence of p_L was 1,447 U (λ PN610), 13-fold greater than that of p_L . The sum of the individual promoter strengths (1,562 U) is similar to their combined measurement of 1,625 U (λ PN538), indicating that the two transcripts neither interfered with nor assisted one another but rather were simply additive.

(ii) cI transcription in the face of p_R activity. In the presence of p_R , lysogenic transcription was reduced 14-fold, from 115 U (λ PN538) to 8 U (λ PN157), while transcription from p_E was reduced twofold, from 1,447 U (λ PN610) to 696 U (λ PN612). Given the additivity of transcription from p_E and p_L observed in the absence of p_R , the expectation was that leftward transcription in the face of converging transcription from p_R would also be greater for the combined activities of p_E and p_L than that found in the presence of p_E alone. It was therefore surprising that leftward transcription fell from 696 U in the ab-

Sal I (22581) p_R Sna BI (23552)							
Leftward						Rightward	
-CII	+CII	p_R	p_L	<i>apl</i>	p_E	-CII	+CII
λ PN78	6 (± 1) 517 (± 43)	+	+	+	+	λ PN117	1278 (± 78) 760 (± 46)
λ PN157	8 (± 1) 444 (± 51)	+	+	-	+	λ PN332	2275 (± 75) 1474 (± 96)
λ PN538	115 (± 15) 1625 (± 41)	-	+	-	+	n.d.	
λ PN610	2 (± 1) 1447 (± 34)	-	-	-	+	n.d.	
λ PN612	2 (± 1) 696 (± 67)	+	-	-	+	n.d.	

FIG. 2. Promoter strengths and interactions. The *Sal*I-*Sna*BI restriction fragment, with the mutations and in the orientations indicated, was cloned in front of the promoterless *lacZ* gene of pMRR9 (13) and transferred as a single copy to the chromosome of MC1061.5 (13) using the reporter system of Simons et al. (25) as previously described (13). The β -galactosidase activity (Miller units) was determined (13) for each of these strains, transformed with either the CII expression plasmid (pPN72)(13) or its *cII*⁻ derivative (pPN178 13). Values shown are the means for six individual cultures of each strain on the same day. Errors are 90% confidence limits determined by the Student *t* test (27). The values determined for four independent single lysogens of the same clone varied by less than 10%. Background activity (1 U) was subtracted from the activities shown and was determined by measuring the β -galactosidase activity of MC1061.5 lysogenized with λ pMRR9 and transformed with either pPN72 or pPN178. Coordinates of the restriction sites refer to the numbers of base pairs from the left end of the chromosome of 186 (GenBank accession number U32222). n.d., not determined.

FIG. 3. Effect of bound CII on rightward transcription. PCR products, generated from wild-type and KS11 (22) templates using primers designed to introduce *Eco*RI and *Kpn*I restriction sites at the left and right ends, respectively, were inserted in front of the promoterless *lacZ* gene of pMRR9 (13) and transferred as single copies to the chromosome of NK7049 (25) as previously described (13). The β -galactosidase activity (Miller units) was determined for each of these strains, transformed with either pPN72 (CII⁺) or the parent plasmid pACYC184 (CII[–]). Values shown are the means for three individual cultures of each strain on the same day, and the errors are 90% confidence limits determined by the Student *t* test (27). The filled box at the start of the *cII* gene represents the CII operator (*O_{CII}*), and the coordinates refer to the number of base pairs from the left end of the chromosome of 186. In our study, the β -galactosidase assays gave values consistently lower for strain NK7049 than for strain MC1061.5.

sence of *p_L* (λ PN612) to 444 U when *p_L* was present (λ PN157). It appeared as if the association of RNA polymerase with *p_L* sensitized transcription from *p_E* to interference by transcription from *p_R*.

(iii) ***p_E* interference with *p_R* activity.** Transcription from *p_E* reduced transcription from *p_R* by 801 U, from 2,275 to 1,474 U (λ PN332). Some of the interference of *p_E* on *p_R* could arise from CII bound at *p_E* acting as a roadblock to the rightward movement of RNA polymerase from *p_R*. To test this possibility, we used the KS11 mutant of *p_E* (22) in which a T-to-C base-pair change in the –10 region eliminates activity of *p_E* (<0.1% of wild type) without altering the DNA binding affinity of CII. In this experiment (Fig. 3), wild-type *p_E* activity caused a 25% reduction in *p_R* activity (618 to 462 U), whereas no reduction was seen with the KS11 mutant. Thus, inhibition of *p_R* by *p_E* is not due to CII blocking the progress of RNA polymerase but requires *p_E* activity.

Conclusions. We have shown that the transcription factor CII elevates 86-fold the transcription of the immunity repressor gene in the face of converging transcription from the lytic promoter, although we have as yet no evidence for the physiological relevance of the CII concentration generated in the present experiments. Furthermore, the elevation of the lysogenic transcription from *p_L* from 8 to 115 U, when the converging transcription from *p_R* is eliminated by mutation, confirms the proposition (5) that repression of the lytic promoter by the immunity repressor would lead to enhanced transcription of the immunity repressor gene. This suggestion not only provides a mechanism for positive feedback in the expression of the immunity repressor but also is consistent with a transient requirement for CII in the establishment of lysogeny. Thus, despite the fundamental difference in switch promoter arrangement, the role of CII in establishing the conditions for positive autoregulation of the immunity repressor is, in principle, the same for 186 and λ . A potential advantage to lysogenization, attributed to the action of the CII-activated transcription from *p_{RE}* after λ infection (20), is the reduction of

lytic transcription from the convergent lytic promoter *p_R*, with the consequent reduction in the synthesis of the anti-immune repressor Cro. In 186, reduced lytic transcription was seen in the presence of active *p_E*, but this proved of no obvious advantage to transcription from the lysogenic promoter *p_L*. In addition, the presence of Apl displayed little impact on the level of *cI* transcription, so that any reduction in Apl synthesis associated with reduced lytic transcription would be of little consequence.

An outstanding goal is to identify the property differentiating P2 from its close relative 186 (4) that enables it to dispense with CII in the establishment of lysogeny. Both phages display frequencies of lysogenization on the order of 10% (2, 4) and both display a face-to-face arrangement of switch promoters, with the lytic promoter at least 100-fold stronger than the lysogenic promoter in the presence of each other and of the anti-immunity gene (1,278 versus 6 U for 186 [Fig. 2] and 38 versus 0.3 chloramphenicol transacetylase unit for P2 [19]). However, while P2 establishes a 10% frequency of lysogeny with this hundred-fold differential in promoter strengths, the use of CII by 186 to establish lysogeny brings the opposing transcripts to near equality (760 versus 517 U) (Fig. 2). The determination of promoter strengths for P2 involved the use of a plasmid reporter vector, and no controls were carried out on the possible variations in plasmid copy number for different constructs. Notwithstanding this qualification and the question of the physiological relevance of CII concentration in the present study, the contrast between 186 and P2 in relative promoter strengths in the establishment of lysogeny between P2 and 186 appears stark. Prophage inducibility is another property, along with the CII requirement, that distinguishes 186 (28) and λ (7) from P2 (2), and the potential interrelationship of prophage inducibility and the CII requirement will be explored in future studies.

Our final comments concern the 14-fold reduction of lysogenic promoter activity in the presence of an active lytic promoter. The lysogenic promoters of both P2 (19) and lambda (8) are also inhibited by the activities of their respective lytic promoters. The transcription start points of the lytic and lysogenic promoters are separated by 62 bp in 186 (5) and by 82 bp in lambda (8). In the case of P2, we predict from the sites of the presumptive –10 regions (18) about a 40-bp separation. As the switch promoters of lambda are arranged back-to-back and the RNA polymerase DNase I footprint overlaps +20 to –55 of an *E. coli* promoter (14), the possibility of RNA polymerase bound at one promoter precluding an RNA polymerase from binding at the second promoter was real, but the interference appears to occur at the open complex formation rather than at the binding step (8). For the face-to-face arrangement of switch promoters of 186 and P2, the probability is that RNA polymerases can occupy both promoters simultaneously. The nature of the interference for these phages has yet to be determined but presumably involves some step subsequent to the binding of the RNA polymerase to the promoter. If interference is due to an elongating RNA polymerase from the lytic promoter dislodging an RNA polymerase slow to clear the lysogenic promoter, then directionality may be important, for we did not find promoter occlusion (1) of *p_L* by transcription from the upstream promoter *p_E*. The nature of promoter interference is currently under study in this laboratory.

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