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Genes for the Establishment and Maintenance of Lysogeny by the Temperate Coliphage 186

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To identify the genes in coliphage 186 that are required for lysogeny, we isolated clear-plaque mutants. Complementation studies and DNA sequencing identified two genes, the *c*I gene for the immunity maintenance repressor and the *c*II gene, which is required only for the establishment of lysogeny. One mutant carried a change in the LexA-binding site controlling expression of the antirepression protein Tum.

The study of temperate phages has led to major insights into a variety of mechanisms of control of gene expression. We are interested in the control of the lysis-lysogeny switch of the nonlambdoid coliphage 186.

186 prophages carrying a *c*Its mutation are temperature inducible (27), showing that the *c*I gene is necessary for the maintenance of lysogeny. The *c*I gene codes for the immunity repressor (7, 13; Fig. 1). The host *lexA* gene is also necessary for maintenance of lysogeny in 186, since the phage *tum* gene, which encodes an antirepression protein, is repressed by LexA (3, 16; Fig. 1). Mutants defining a second gene necessary for lysogeny, *c*II (11), have not been characterized. The 186 *int* (integrase) gene (13) is required for the establishment of stable lysogeny, but it is not required for its maintenance (2). 186 *int* mutants form turbid plaques and therefore, like λ *int* mutants, are capable of establishing abortive lysogeny (9, 20).

We have now undertaken a systematic isolation and characterization of clear-plaque mutants to define the genes required for lysogeny.

Clear-plaque mutants define four complementation groups. Independent clear-plaque mutants were isolated following UV mutagenesis of wild-type 186 (186^+) (12) as described by Lamont et al. (17), except that mutagenized phage was plated directly onto strain 594 (*sup*° *strA*) (4). The isogenic nonsuppressing and suppressing strains of Garen et al. (8) were used to test for amber mutations. Complementation tests were done using the cross-streak technique (18) on 594 on T plates (10). One mutant, which was unable to complement *c*Its_p (1, 27) and was amber suppressible, was designated *c*Iam461. The other mutants were then tested for their ability to complement 186*c*Iam461 (or 186*c*Its_p) and 186*c*II408 (a nonsuppressible *c*II mutant [11]). The 31 new mutants fell into four complementation groups: there were 13 *c*I mutants, 16 *c*II mutants, 1 *c*III mutant, and 1 *c*IV mutant.

Sequencing of *c*I mutations. DNA sequencing (19, 25) of the *c*I gene in the *c*Iam461 mutant revealed two changes from the *c*Its_p sequence (13; Fig. 1): a C→T change at sequence position 2675 (2675:C→T), giving an amber codon at codon 8 of the *c*I gene (CI:W8→am), and a 2634:T→C change. Since the *c*Iam461 mutant was isolated from wild-type phage, the *c*Its_p mutation is a 2634:C→T change (CI: G22→R). The *c*I10 mutant (a nonsuppressible *c*I mutant [11]) carries a frameshift mutation in *c*I caused by replacement of an A and a T at positions 2370 and 2371, respectively, with a single G (22).

Identification of the *c*II gene. The *c*II408 mutation was located between the unique *Xho*I.629 and *Bgl*II.4244 sites in 186 DNA by an in vitro reconstruction experiment (16). Ligation of the 26.4-kb *Bgl*II.4244-*Xho*I.629 fragment from 186^+ DNA with the 3.6-kb *Xho*I.629-*Bgl*II.4244 fragment from *c*II408 DNA gave clear plaques after transfection. The reciprocal ligation gave turbid plaques. The *Xho*I.629-*Bgl*II.4244 region is likely to encode eight genes (13, 24; Fig. 1). A deletion mutant, Δ1, lacking *CP69*, *int*, and most of the *c*I gene (13), was able to complement the *c*II408 mutation. Phages which have mutations in *fil*, *dhr*, or *CP79* form turbid plaques (23), as do phages carrying an in-frame deletion in the *apl* gene (5). To test whether *CP76*, the last remaining gene candidate on the *Xho*I.629-*Bgl*II.4244 fragment, was the *c*II gene, an amber mutation was created by oligonucleotide mutagenesis (23) of a clone of the wild-type *Sac*I.1403-*Bgl*II.4244 fragment in the *Sac*I and *Bam*HI sites of M13tg131 (14). The change was 3343:C→T, producing a CP76:Q75→am change. The mutation was checked by sequencing and was transferred into 186 by the triple fragment ligation of the *Sna*BI.3238-*Xho*II.4244 fragment from the M13 clone with the *Xho*I.629-*Sna*BI.3238 fragment from 186*c*Its_p and the large *Bgl*II.4244-*Xho*I.629 fragment from 186^+ followed by transfection to give the reconstituted phage as a plaque. On the basis of plaque morphology on nonsuppressor and suppressor strains and complementation tests, the reconstituted phage behaved as a *c*IIam mutant, identifying *CP76* as the *c*II gene. This mutation was named *c*IIam475 (Fig. 1).

The *c*III mutation. The *c*III464 mutation was mapped outside the *Xho*I.629-*Bgl*II.4244 region by in vitro reconstruction, as described above. The mutation was then similarly mapped between the unique *Not*I.89% site and *cos* (16) and finally located between the *Pst*I.94% and *cos* sites. The nucleotide sequence between *Pst*I.94% and *cos* has been determined (15), but beyond *Bam*HI.96% (25) it has not yet been published. It contains two genes, the *tum* gene and *CP97*, whose transcription from the P_{95} promoter is under LexA control (16). The only change in the nucleotide sequence of the *c*III464 mutant was the deletion of a T at position 4496 of the sequence reported by Sivaprasad et al. (25). The mutation is in the predicted LexA box spanning P_{95} (Fig. 1) and completely destroys its similarity to known LexA-binding sites (26).

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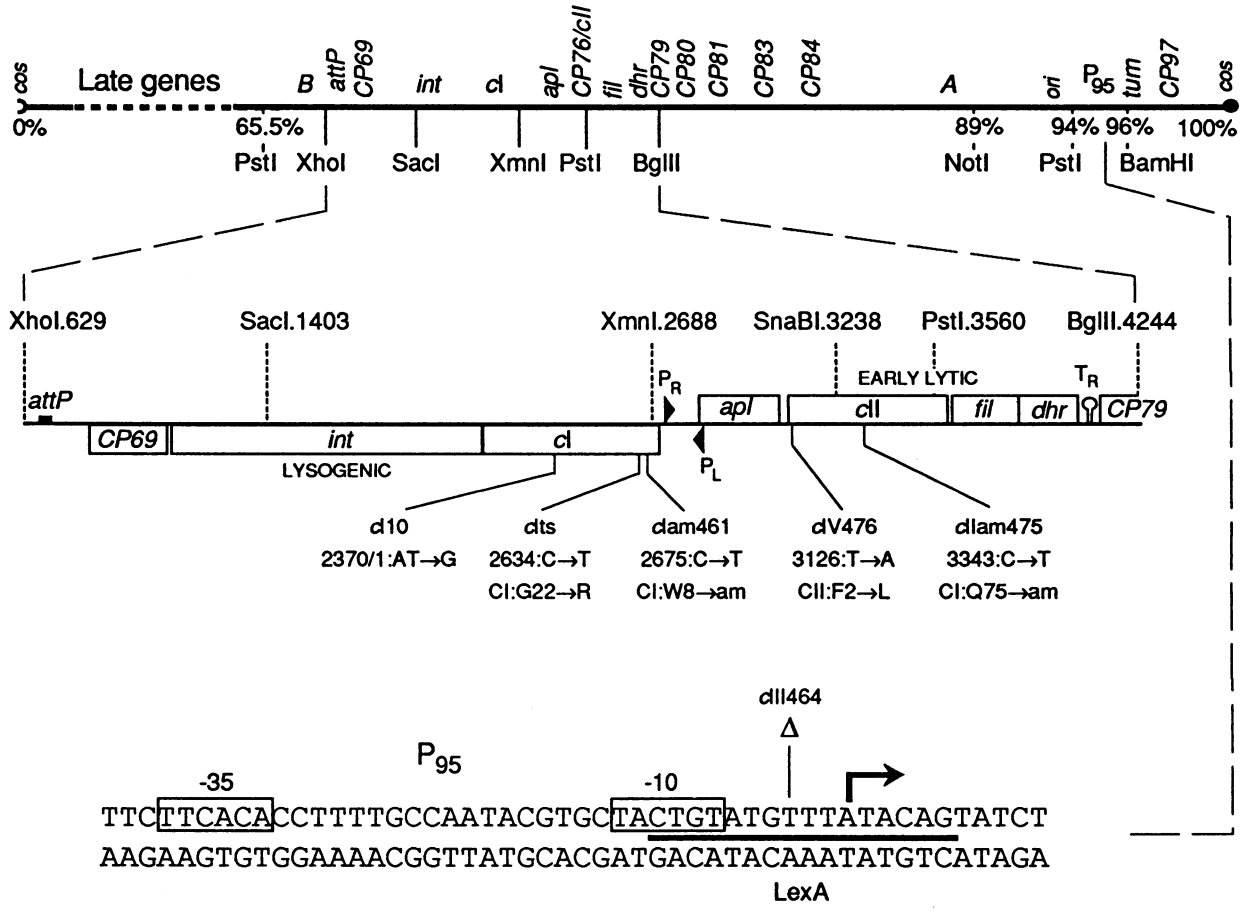

FIG. 1. Location of clear-plaque mutations. The upper part of the figure is a map of the 186 chromosome, showing the genetic constitution of the early region and relevant restriction sites (13, 15, 24, 25). Genes with the prefix *CP* are of unknown function. The central part of the figure shows the *Xho*I.629-*Bgl*II.4244 region (7, 13, 23, 24) in more detail, with the positions of clear-plaque mutations indicated. Genes are indicated by boxes (rightward genes above the line, leftward genes below). The promoters P_R and P_L are shown by solid arrowheads. The sequence numbering begins at the *Pst*I site at 65.5% and extends rightward. All mutations are listed here and in the text as changes to the *l* (top) strand of the wild-type sequence. Restriction sites are specified by the sequence position before the cleavage point. The lower part of the figure shows the sequence of P_{95}, which is the promoter for *tum* and *CP97*. The in vitro transcription start site (21) is indicated by a thick bent arrow. The putative LexA-binding site (14 of 16 match with consensus [26]), involved in *tum* repression (16), is denoted by a line between the sequence strands. The *c*III464 mutation (a deletion of one of the three T residues) is shown.

Thus, the *c*III464 mutation does not appear to define a gene. The clear-plaque phenotype can be explained by a presumed loss of LexA repression of the *tum* antirepression gene. However, further studies are needed to characterize this operon, particularly the control of its expression, and at that time a better understanding of the *c*III464 mutant will be pursued.

The *c*IV mutation. The *c*IV476 mutation was mapped to the *Xho*I.629-*Bgl*II.4244 region. It was then mapped to the left of the *Pst*I.3560 site by using the *Xho*I.629-*Pst*I.3560 and *Pst*I.3560-*Bgl*II.4244 fragments from 186^+ or *c*IV476 and the large *Xho*I-*Bgl*II fragment from 186^+. The *c*IV476 mutant complemented Δ1, indicating that the *c*IV mutation must lie to the right of the start of the *c*I gene at position 2697. The *Xmn*I.2688-*Pst*I.3560 fragment from *c*IV476 was cloned into M13, and the *apl-c*II region was sequenced by using a set of primers. The only change from the wild-type sequence was a 3126:T→A change, giving an F→L change in the second amino acid of CII (Fig. 1). This was unexpected, since *c*IV476 was able to complement a number of *c*II mutants, including *c*IIam475, and thus this may be an example of intragenic complementation. The *c*IV mutant may produce a mutant CII polypeptide that is able to interact with other mutant CII polypeptides to form functional CII multimers.

The *c*II gene is not necessary for the maintenance of lysogeny. The location of the *c*II gene in the P_R lytic operon, which is repressed by CI during lysogeny (7), suggests that *c*II is not necessary to maintain lysogeny. The results of two experiments support this.

The free 186 phage arising by spontaneous prophage induction in cultures of 186^+ lysogens are turbid-plaque formers. However, in *recA*(186^+) cultures the free phage titer is severely reduced and the phages are predominantly clear-plaque formers (27). In the absence of the RecA-LexA-Tum prophage induction pathway (16), spontaneous mutation of phage genes involved in the maintenance of lysogeny becomes a major means of prophage induction. We tested 30 clear-plaque formers from the supernatant of a *recA*(186^+) culture, and all were cI^- cII^+, suggesting that *c*I is the only phage gene needed for the maintenance of lysogeny.

Mutants *c*II408, *c*II439, and *c*IV476 gave slightly turbid plaques, and stable 594 lysogens of these mutants could be

obtained. The spontaneous prophage induction rates of these lysogens, as judged from free phage levels, were only slightly higher than that of the 186^+ lysogen (data not shown), suggesting that the *c*II gene is not required for the maintenance of lysogeny.

The predicted CII protein contains a likely helix-turn-helix DNA-binding motif (6) (residues 15 to 36), so we expect that CII is a transcriptional regulator. Experiments are in progress to characterize the *c*II gene product and its role in establishing lysogeny.

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