

Autoregulation of bacteriophage P2 repressor

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The immunity repressor of bacteriophage P2 regulates the two convergent promoters, P_e and P_c, located in the early control region. P_e is the early promoter which is negatively regulated by the repressor. It was found, by DNase I protection studies, that the P2 repressor enhances the binding of RNA polymerase to P_c. Furthermore, under *in vivo* conditions the transcription initiated at P_c, measured as chloramphenicol acetyl transferase gene expression, is low in the absence of repressor but is stimulated by low repressor levels. With increasing repressor concentrations transcription from the P_c promoter decreases. Thus, the P2 repressor both negatively and positively regulates its own promoter.

Key words: Bacteriophage/regulation/repressor/activator/promoter

Introduction

The lysogenic state of temperate phages is promoted and maintained by a regulatory protein, the repressor. Most of our knowledge of repressor structure and function comes from studies of phage lambda and its relatives. The immunity repressor of phage lambda, the *ci* gene product, is a polypeptide that is 236 amino acids long. It has two domains and binds as a dimer to three individual sites in each of two operator regions (Johnson *et al.*, 1981). In addition, lambda has an antirepressor of 66 amino acids, the *cro* gene product, which binds to the same three sites in the right operator region (Johnson *et al.*, 1981).

P2 belongs to a group of temperate, non-inducible phages unrelated to phage lambda. The P2 repressor, coded for by the *C* gene, is a polypeptide of 99 amino acids. It binds to only one operator region which regulates the expression of the early genes: *cox*, *B* and possibly *A*. Previous studies have failed to yield any evidence for the existence of a P2 antirepressor (Nilsson and Bertani, 1977), and rather little is known about the regulation of the repressor level.

The fact that repressor binding activity is not strictly proportional to the number of repressor gene copies present in a P2 lysogenic host (Lundqvist and Bertani, 1984) suggests that autoregulation may be involved in repressor synthesis. To test this hypothesis we have studied the effect of repressor on RNA polymerase binding to the repressor promoter (P_c), and also determined the effect of repressor on transcription initiated at P_c under conditions where P_c controls the expression of CAT (chloramphenicol acetyl transferase).

Results

Foot-printing analysis of the binding of RNA polymerase to the P2 repressor promoter

The repressor gene, *C*, is transcribed from right to left on the genetic map, i.e. in the opposite orientation to the early operon (see Figure 1). Therefore the region between the coding parts of the *C* gene and the first gene of the early operon, *cox*, which is only ~110 bp long, should contain the promoters for the early operon (P_e) and for the repressor (P_c) as well as the operator region.

The location of P_e and P_c can be inferred from the DNA sequence (Ljungquist *et al.*, 1984) (see Figure 1), and the transcripts initiated at the respective promoter would be overlapping for about 30 nucleotides. To test this we performed DNase protection studies to locate the site of binding of RNA polymerase. Figure 2A shows that RNA polymerase binds to the P_e and P_c regions. The fact that the -10 and -35 regions of P_e were fully protected, whereas those of P_c were only partially protected, indicates that the binding of RNA polymerase to P_e is much stronger than its binding to P_c under comparable conditions.

DNA sequence analysis of virulent P2 mutants has indicated the location of the repressor binding sites as direct repeats of eight nucleotides separated by 14 nucleotides, O1 and O2 (Ljungquist *et al.*, 1984) (see Figure 1). This prediction was verified by the DNase protection studies; Figure 2B shows that the repressor protects several regions around the -10 region of P_e. In between these protected regions, some residues show enhanced cleavage.

To test the hypothesis that the immunity repressor regulates its own synthesis, the binding of RNA polymerase to the P_c region was studied by DNase protection studies in the presence or absence of purified P2 repressor. As can be seen in Figure 3 (lanes 6–9), the addition of repressor protein alone did not affect the DNase I cleavage pattern of the P_c region. The region protected by the repressor ends at the bottom of the figure where some residues were fully protected, while others showed enhanced cleavage (lanes 6 and 9). In the presence of repressor, RNA polymerase binding to the P_c region was enhanced as the cleavage pattern over P_c was changed (lanes 1 and 2). Several bases were protected while others showed enhanced cleavage. In fact, in the absence of repressor, RNA polymerase bound poorly or not at all to P_c under the conditions used. Thus, under these *in vitro* conditions RNA polymerase binding to P_c seems to be enhanced by repressor. It cannot, however, be excluded that this effect is due to a contaminant in the repressor preparation, but the *in vivo* experiments described below seem to exclude this possibility.

Construction of a plasmid where CAT expression is under control of the P2 repressor promoter

Previous experiments have indicated that the 76.7–79.7% (*Pst*I–*Eco*RI) region of the P2 genome, which contains part of

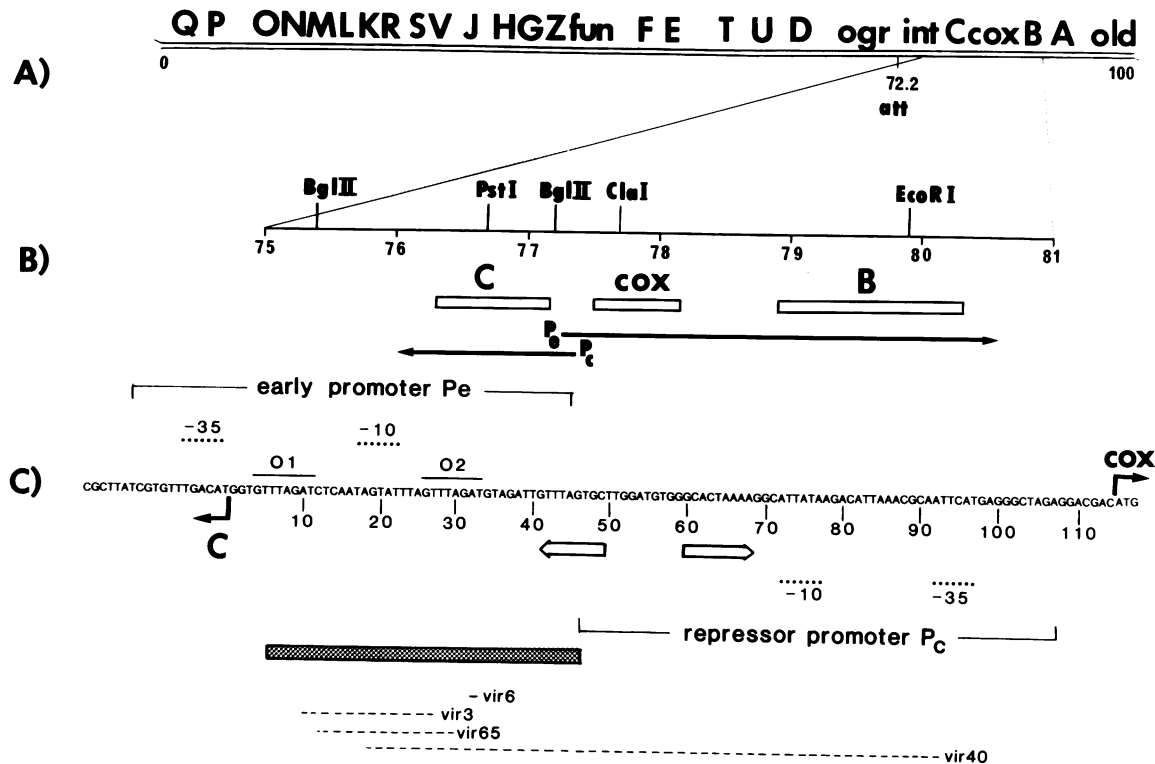


Fig. 1. Map of phage P2. (A) The gene sequence of the whole genome is shown above the line. (B) Enlargement of the 75–81% region. The restriction sites used for the plasmid constructions are indicated above the line, and the location of the genes are indicated by the boxes below the line. The initiation codons of genes *C* and *cox* are indicated by the arrows. The promoter regions with the respective -10 and -35 regions are indicated by the dotted bars. The two direct repeats in the operator region (Ljungquist *et al.*, 1984) are indicated by the arrows O1 and O2. The repressor binding region as determined by DNase I protection studies is indicated by the shaded bar below the line. The open arrows below the line indicate the inverted repeat. The interrupted lines below the shaded bar indicate the deletions in the virulent mutants. Note that the ends of the *vir3* deletion are located in a direct repeat. Therefore, its exact ends cannot be determined.

the *C* gene, all of gene *cox* and part of the *B* gene (see Figure 1), cannot be cloned into plasmid pBR322. This seems to be due to a lethal effect of a small polypeptide, encoded by a gene that is located between gene *cox* and gene *B* (unpublished). Therefore, if the early transcription initiated at P_e is blocked by the P2 repressor, the 76.7–79.9% region can be cloned into plasmid pBR322.

To obtain a plasmid where CAT is under the control of P_c under non-immune conditions the following constructions were made (see Figure 4A). The 76.7–79.9% (*Pst*I–*Eco*RI) fragment of phage P2 was inserted into the *Bam*HI site of the plasmid vector pKK232-8 (Brosius, 1984). After transformation into the lysogenic strain C-117, ampicillin-resistant clones containing P2 hybridizable material were isolated. The recombinant plasmids thus obtained were analyzed by restriction enzyme cleavages (data not shown). One clone in which the P2 insert was oriented such that CAT is under the control of P_c was isolated for further work – plasmid pSS14-24. The region between the *Cla*I and the *Sma*I sites, containing the genes of the P_e operon, was removed and the DNA was recircularized and transformed into the non-lysogenic strain C-1a, selecting for ampicillin resistance. One clone, carrying plasmid pSS31-2 was selected for further studies (see Figure 4A).

Transcription from the P2 repressor promoter in P2 sensitive and immune cells

As the DNase protection studies described above indicated that the P2 repressor activates P_c , plasmid pSS31-2 was transferred

into the P2 lysogenic strain C-117, selecting for ampicillin-resistant clones. Strain C-117 carrying pSS31-2 was found to grow better on plates containing 50 μ g chloramphenicol/ml than strain C-1a carrying the same plasmid.

To compare the expression of the CAT gene contained on plasmid pSS31-2 in the sensitive strain C-6001 and the P2 lysogenic strain C-6003, the acetylation of [14 C]chloramphenicol by cell extracts was measured by silica gel t.l.c. (Gorman *et al.*, 1982). As can be seen in Figure 5, the conversion of chloramphenicol to acetylated derivatives per μ g protein was higher in the extract obtained from the P2 lysogenic strain C-6003 compared to the P2 sensitive strain C-6001.

Expression of the P2 repressor promoter at different immunity levels

To study the correlation between the activity of P_c and the immunity level, plasmid pSS31-2 was transferred to cells having different immunity levels. The efficiency of plating of certain P2 virulent mutants is affected by repressor gene dosage; i.e. P2*vir6* will only plate on a single lysogen, while *vir3* will plate on double and triple lysogens as well (Bertani and Bertani, 1971; Westöo and Ljungquist, 1980). To obtain cells with a higher immune level than that obtained in a triple lysogen, the P2 repressor gene was cloned into pACYC177 which is compatible with the *ColE1*-like replicon of pBR322. Plasmid pSS17-7 is such a pACYC177 derivative which contains four copies of the P2 repressor gene (See Materials and methods). The immunity levels of the transformed cells were tested by titration of the P2 mutants

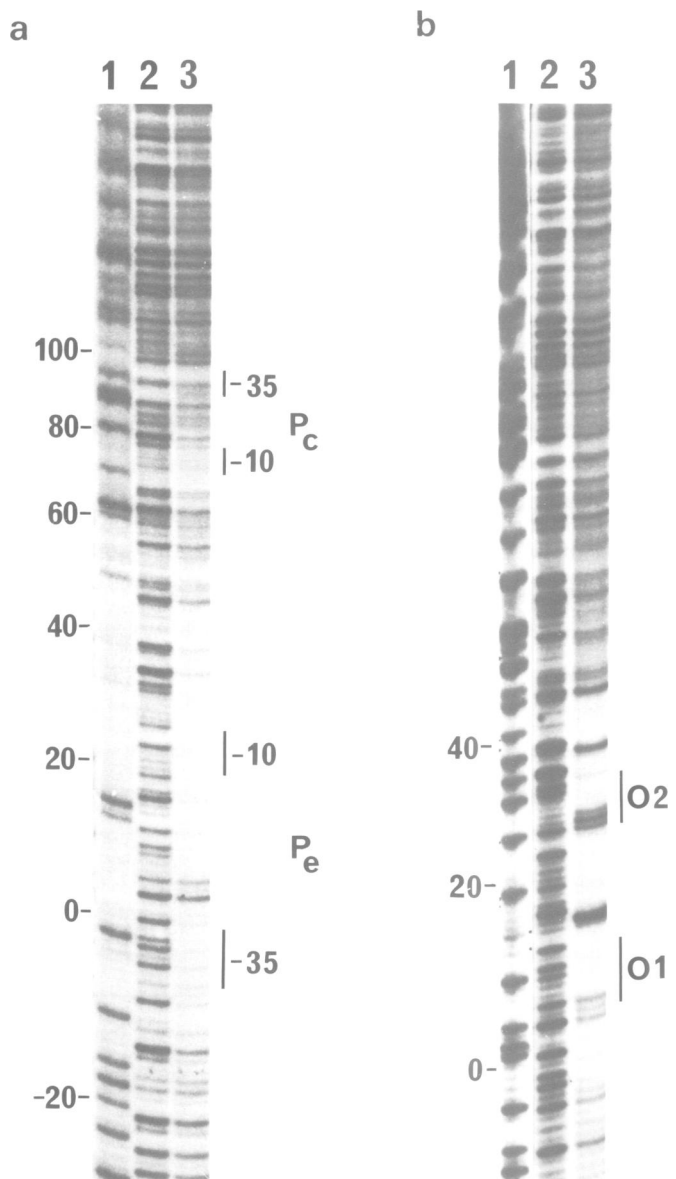


Fig. 2. DNase I footprints of RNA polymerase and P2 repressor to the early control region. (a) RNA polymerase binding. The location of the -10 and -35 regions of P_e and P_c are indicated. **Lane 1**, G reaction; **lane 2**, no addition; **lane 3**, 20 units RNA polymerase. (b) Repressor binding. The location of the direct repeats, O1 and O2, are indicated. **Lane 1**, C reaction; **lane 2**, No addition; **lane 3**, 50 μ l repressor. The repressor preparation contained 0.1 μ g repressor/ μ l.

vir6, *vir3*, *vir40* and *vir65*. As can be seen in Table I, the presence of pSS31-2 did not affect the plating efficiencies of the virulent mutants to any large extent. Furthermore, the single lysogenic strain only inhibited the plating of *vir6*, but the double and triple lysogenic strains inhibited the plating of *vir6* as well as of *vir3* as shown previously (Westöo and Ljungquist, 1980). Cells harbouring plasmid pSS17-7 showed a higher immunity level than the triple lysogenic strain as the plating efficiency of *vir40* was reduced. The *vir65* mutant plated on all strains with equal efficiency, and therefore seems to be totally immunity insensitive.

The CAT activities of extracts obtained from the transformed cells described above was measured. As can be seen in Table I, the CAT activity in the transformed P2 sensitive strains C-6001

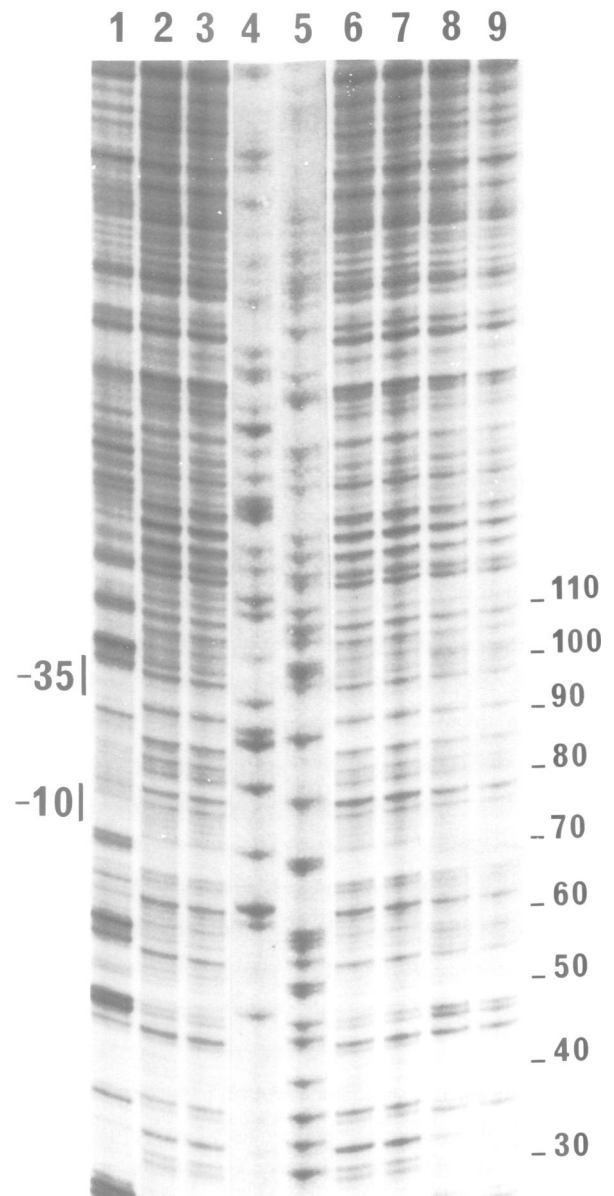


Fig. 3. DNase I footprints of the binding of RNA polymerase to P_c in the presence or absence of repressor. The -10 and -35 regions of P_c are indicated. **Lane 1**, 20 units RNA polymerase and 5 μ l partially purified repressor; **lane 2**, 20 units RNA polymerase; **lane 3**, 5 units RNA polymerase. **lane 4**, G reaction; **lane 5**, C reaction; **lane 6**, No addition; **lane 7**, 0.2 μ l repressor; **lane 8**, 1 μ l repressor, **lane 9**, 5 μ l repressor. The numbers refer to the base number from the initiation codon of the C gene of the G and C reactions. The repressor preparation contained 2–3 μ g repressor/ μ l.

and K-501 was fairly low. In the single lysogenic derivatives, C-6003 and K-503, CAT activity was increased 4- to 5-fold. Increasing the repressor level above the level of a single lysogen reduced the CAT expression, but even at the high immunity level obtained in cells harbouring the plasmid pSS17-7 the reduction was only ~50%. This decrease was not due to a reduction in the copy number of plasmid pSS31-2 caused by the presence of plasmid pSS17-7, since single-cell resistance to penicillin mediated by plasmid pSS31-2 in P2 lysogenic strain was not affected by the presence of plasmid pSS17-7; single-cell resistance to penicillin is proportional to the gene dosage (copy number) of the β -lactamase gene (Uhlén and Nordström, 1977).

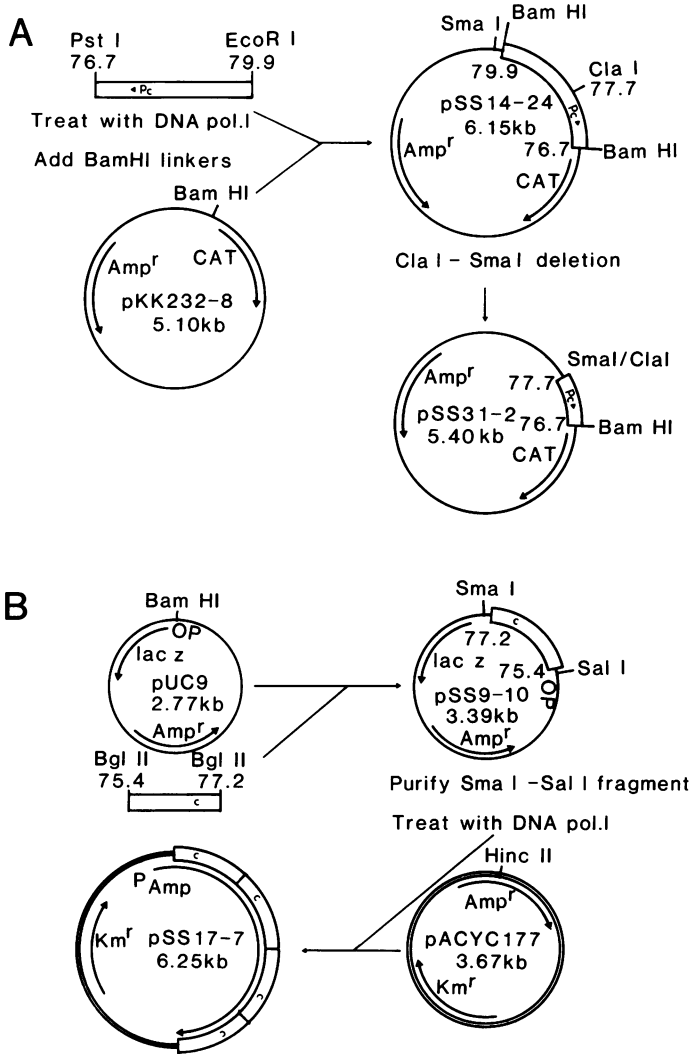


Fig. 4. (A) Plasmid pSS31-2 was constructed from pSS14-24 by deleting the *Cla*I-SmaI fragment [in order to make the *Cla*I site accessible, a *Dam*⁻ host, GM33, was used (Marinus and Morris, 1974)]. The DNA was treated with the Klenow fragment of DNA polymerase I, purified by agarose (1%) gel electrophoresis. The proper fragment was eluted, ligated and transformed into strain C-1a, selecting for ampicillin resistance. (B) Plasmid pSS17-7 was constructed in two steps. First the *Bgl*II 75.4%–77.2% fragment of P2 wild-type was inserted into the *Bam*HI site of plasmid pUC9 to give plasmid pSS9-10. Then the *Sma*I-SalI fragment of plasmid pSS9-10 was isolated, treated with DNA polymerase I and inserted into the *Hinc*II site of plasmid pACYC177.

Discussion

The early control region in phage P2 contains two convergent promoters; *P_c* which controls the early operon and *P_c* which controls repressor synthesis. The -10 hexamer of *P_c* is precisely homologous to the consensus sequence of an *Escherichia coli* promoter, but the location of -35 region is not as evident. However, with a spacing of 14 nucleotides the sequence ATGAAT, which has two of the three conserved residues of the consensus -35 region, can be found (see Figure 1). Our DNase protection analyses of RNA polymerase binding to *P_c* show that repressor enhances RNA polymerase binding to *P_c* *in vitro* (see Figure 3). The *in vivo* analyses of the present study, where CAT expression is controlled by *P_c*, demonstrate the activation of *P_c* by the repressor (see Figure 5 and Table I). Hence, the P2 repressor promoter *P_c* appears similar in mode of activity to other promoters with poor homology to the -35 region consen-

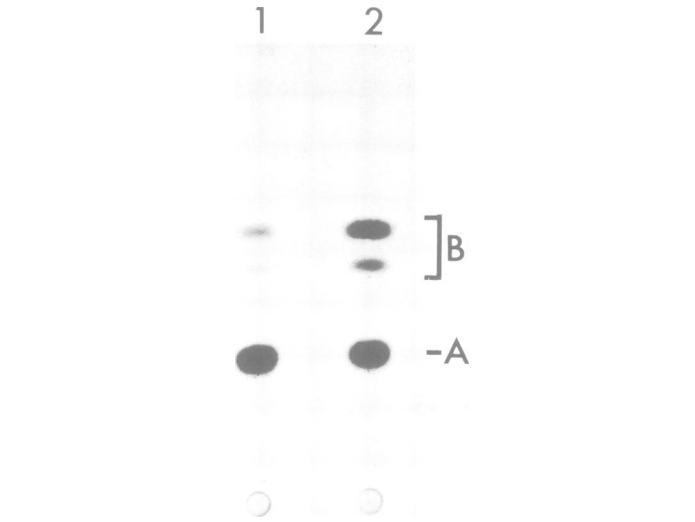


Fig. 5. Assay of CAT activity. The bacteria were grown in LB medium (Bertani, 1951) containing 50 μ g ampicillin or kanamycin per ml to a cell density of $\sim 3 \times 10^8$ /ml. Ten ml were washed and concentrated in 4 ml 100 mM Tris-HCl, pH 7.9. The cells were disrupted by sonication and the extracts were diluted 20-fold and assayed for CAT activity by following the acetylation of [¹⁴C]chloramphenicol (Gorman *et al.*, 1982). On the autoradiograph of the silica gel, the locations of chloramphenicol (A) and 1-acetate chloramphenicol and 3-acetate chloramphenicol (B) are indicated. Lane 1, The non-lysogenic strain C-6001; lane 2, the single lysogenic strain C-6003. The protein concentrations of the extracts were in C-6001, 430 μ g/ml and in C-6003, 590 μ g/ml.

Table I. Efficiencies of plating of P2 virulent mutants on various bacterial strains and the expression of the repressor promoter *P_c* at different levels of immunity

Strain	Prophage or plasmid carried	Plating efficiencies of infecting phage ^a				Relative CAT activity ^b
		<i>vir6</i>	<i>vir3</i>	<i>vir40</i>	<i>vir65</i>	
C-1a	-	1	1	1	1	ND
C-6001	pSS31-2	1	1	1	1	1.5
C-6002	pSS17-7	< 10 ⁻⁴	< 10 ⁻⁴	0.05 ^d	1	ND
C-117	1 P2	< 10 ⁻⁴	1	1	1	ND
C-6003	1 P2 + pSS31-2	< 10 ⁻⁴	1 ^c	1	1	7.7
C-6004	1 P2 + pSS31-2 + pSS17-7	< 10 ⁻⁴	0.1 ^d	0.2 ^d	1	3.5
K-234	-	1	1	1	1	ND
K-501	pSS31-2	1	1	1	1	1.1
K-502	pSS17-7	< 10 ⁻⁴	< 10 ⁻⁴	< 10 ⁻⁴	1	ND
K-243	1 P2	< 10 ⁻⁴	1 ^c	1	1	ND
K-503	1 P2 + pSS31-2	< 10 ⁻⁴	1 ^c	1	1	4.3
K-244	2 P2	< 10 ⁻⁴	0.05 ^d	1	1	ND
K-504	2 P2 + pSS31-2	< 10 ⁻⁴	0.05 ^d	1	1	3.9
K-246	3 P2	< 10 ⁻⁴	< 10 ⁻⁴	1	1	ND
K-505	3 P2 + pSS31-2	< 10 ⁻⁴	0.01 ^d	1	1	3.5
K-506	1 P2 + pSS31-2 + pSS17-7	< 10 ⁻⁴	< 10 ⁻⁴	0.5 ^d	1	1.8

^aAt 30°C

^bThe CAT activity was determined as described in Figure 5. The labelled spots were cut out and counted. The CAT activity was calculated from the ratio between the c.p.m. in acetylated chloramphenicol and the total c.p.m. and normalized to 100 μ g of protein/ml.

^cSmall plaques

^dExtremely small plaques ND = not determined.

Table II. Bacterial strains, phages and plasmids

Designation		Origin or reference
<u>Derivatives of <i>E. coli</i> strain C</u>		
C-1a	Prototrophic	Sasaki and Bertani (1965)
C-117	C-1a lysogenized with P2	Bertani (1968)
C-6001	C-1a transformed with plasmid pSS31-2	This paper
C-6002	C-1a transformed with plasmid pSS17-7	This paper
C-6003	C-117 transformed with plasmid pSS31-2	This paper
C-6004	C-117 transformed with plasmids pSS31-2 and pSS17-7	This paper
<u>Derivatives of <i>E. coli</i> strain K-12</u>		
K-234	<i>endA1, supE44, thi-1, λ^r, his-211, hsd-16, recA13</i>	Hudnik-Plevnik and Bertani (1980)
K-243	Single lysogen, K-234 (P2 <i>amp137 del1 tsD4</i>)	Lundqvist and Bertani (1984) ^a
K-244	Double lysogen, K-243 (P2 <i>del1 tsD4</i>)	Lundqvist and Bertani (1984) ^a
K-246	Triple lysogen, K-244 (P2 <i>ama127 del1</i>)	Lundqvist and Bertani (1984) ^a
K-501	K-234 transformed with plasmid pSS31-2	This paper
K-502	K-234 transformed with plasmid pSS17-7	This paper
K-503	K-243 transformed with plasmid pSS31-2	This paper
K-504	K-244 transformed with plasmid pSS31-2	This paper
K-505	K-246 transformed with plasmid pSS31-2	This paper
K-506	K-243 transformed with plasmids pSS31-2 and pSS17-7	This paper
GM33	F ⁻ <i>dam-3</i>	Marinus and Morris (1974)
P2 <i>vir3</i>	17-bp deletion in the operator region	Bertani (1957) Ljungquist <i>et al.</i> (1984)
P2 <i>vir6</i>	1-bp deletion in the operator region	Bertani (1957) Ljungquist <i>et al.</i> (1984)
P2 <i>vir40</i>	75-bp deletion in the operator region	Bertani (1957) Haggård-Ljungquist, unpublished
P2 <i>vir65</i>	17-bp deletion in the operator region	Bertani (1957) Haggård-Ljungquist, unpublished
pKK232-8	Ampicillin-resistant pBR322 derivative containing a promoterless CAT gene	Brosius (1984)
pSS14-24	A pKK232-8 derivative with the 76.7–79.9% region of P2 wild-type integrated counterclockwise into the <i>Bam</i> HI site	This paper
pSS31-2	Deletion derivative of pSS14-24 with only the 76.7–77.7% region of P2.	This paper
pACYC177	A P15 derivative	Chang and Cohen (1978)
pSS17-7	A pACYC177 derivative with four copies of P2 gene <i>C</i> integrated into the <i>Hinc</i> II site, so that three <i>C</i> gene copies are read from the ampicillin promoter and one in the opposite orientation. It expresses high levels of P2 repressor.	This paper
pUC9	Ampicillin-resistant pBR322 derivative containing the <i>lacZ</i> gene of <i>E. coli</i> .	Vieira and Messing (1982)
pSS9-10	A pUC9 derivative with the 75.4–77.2% region of P2 wild-type integrated into the <i>Bam</i> HI site in the counterclockwise orientation.	This paper

^aIn Lundqvist and Bertani (1984), where the derivation of these strains from K-234 is described, there is an error in the genetic structure given for K-234. All these strains are *recA13*.

sus hexamer since they frequently are controlled by activators (Raibaud and Schwartz, 1984).

Approximately 100 repressor dimer copies are found in a cell lysogenic for P2 (Lundqvist and Bertani, 1984). The results presented here show one way by which the upper level of repressor concentration can be controlled, as an increase of the repressor concentration above the level normally present in a single lysogen leads to a reduction of the transcription initiated at P_c (see Table I). But, even at high repressor concentrations, as presumably obtained by cloning the repressor gene into pACYC177, P_c is still more active than it is in the absence of repressor (see Table I). It is possible that under the conditions used, the repressor level is not high enough to turn off P_c.

The bifunctional role of the P2 repressor is very similar to the lambda *cI* repressor which controls its own promoter as an activator at low concentrations, but at high concentrations it acts like a repressor (Ptashne *et al.*, 1980). In phage lambda it seems as if a direct contact between RNA polymerase and the *cI* repressor is involved in the activation of lambda P_{RM} (McClure, 1985). In the case of activation of P_c by the P2 repressor an alternative explanation is available. In the control region there is an eight-nucleotide-long inverted repeat (indicated by the open arrows in Figure 1) which overlaps with the repressor promoter P_c. The repressor binding region, as determined by DNase protection studies covers part of the left arm of the inverted repeat (see Figure 1). Hence, in the presence of repressor, the P_c promoter will be fully free to interact with RNA polymerase, but under non-immune conditions the putative loop only allows a limited access of RNA polymerase to the P_c promoter.

Materials and methods

Biological materials

For bacterial strains, phages and plasmids see Table II.

Chemicals and enzymes

[¹⁴C]chloramphenicol, [³²P]ddATP and deoxynucleotide transferase were purchased from Amersham. Restriction enzymes were from Boehringer-Mannheim or New England Biolabs. *E. coli* RNA polymerase was from Boehringer-Mannheim, and DNase I from Sigma.

Preparation of end-labelled DNA fragment

P2 DNA was cleaved with *Pst*I, and the 3'-end was labelled with terminal deoxynucleotide transferase and [³²P]ddATP. The labelled fragments were recleaved with *Nde*I and the fragments were separated by gel electrophoresis. The 76.7–83.4% fragment was located under u.v. light, cut out, sliced and eluted by diffusion in 10 mM Tris, 1 mM EDTA, 0.3 M NaCl, pH 7.5 at 37°C for at least 48 h. The DNA was collected on a DEAE column, eluted and concentrated by ethanol precipitation.

DNase I protection experiments

Thirty-five nanograms of the P2 DNA restriction fragment (76.7–83.4%), labelled at the 3'-end of the *Pst*I cleavage site at 76.7% on the P2 map, was incubated with P2 repressor, partially purified by phosphocellulose chromatography as described in Lundqvist and Bertani (1984) and/or RNA polymerase in a total volume of 100 μl. The repressor preparation contained at least 50–80% repressor protein. Final buffer composition was 50 mM Tris-HCl, pH 8.0, 80 mM KCl, 8 mM MgCl₂, 3 mM CaCl₂, 0.2 mM EDTA, 2 mM DTT, 10% glycerol and 100 μg BSA/ml (lanes 1–3) or 17 mM Tris-HCl, pH 7.4, 0.1 M NaCl, 5 mM MgCl₂, 0.2 mM EDTA, and 12.5% glycerol (lanes 6–9). After 15 min at 37°C, 4 ng of DNase I was added and incubation continued for 1 min. When incubated with both repressor and RNA polymerase, DNA was first incubated with repressor for 15 min, then RNA polymerase was added and incubation was continued for another 15 min before DNase I treatment. The reaction was stopped by transferring the reaction mixture to an equal volume of a stop solution in ice containing 0.6 M NaOAc, 100 mM EDTA, 12.5 μg tRNA/100 μl. Samples were immediately extracted once with an equal volume of phenol saturated with TE buffer (0.02 M Tris, pH 7.5, 0.002 M EDTA) followed by ether extractions and ethanol precipitation. The ethanol-washed pellet was dissolved in gel loading buffer. Equal amounts of radioactivity were applied to an 8% DNA sequencing polyacrylamide gel 0.4 × 230 × 1000 mm in size along with sequencing reaction samples (G and C) to serve as position markers. DNA sequencing was done with the method

of Maxam and Gilbert (1980). The gel was autoradiographed using Kodak Xomat XRP1 film.

Construction of pSS17-7 (see Figure 4B)

Plasmid pKK232-8 has a ColE1 replicon. Therefore, we wanted to have the repressor cloned into plasmid pACYC177, which is compatible with the ColE1 replicon. The P2 *Bgl*III 75.4–77.2% fragment contains the whole coding part of the P2 repressor gene *C*, but lacks the P_c promoter (Westöo and Ljungquist, 1980) (see Figure 1). The 75.4–77.2% fragment was first inserted into the *Bam*HI site of plasmid pUC9 (Vieira and Messing, 1982), selecting for Lac⁻Ap^R transformed cells. From one clone (pSS9-10) which had the P2 *Bgl*III fragment integrated in the clockwise orientation, the P2 fragment was isolated by cleavage with *Sma*I and *Sal*I. In this way a termination codon* is added before the initiation codon of the repressor gene, which ensures that a hybrid protein is not generated after integration of the *Sma*I–*Sal*I fragment into the *Hinc*II site of plasmid pACYC177. Kanamycin-resistant, ampicillin-sensitive recombinant clones were isolated after transformation into strain C-1a and tested for a high immunity level by titration against P2 virulent mutants. One clone, pSS17-7, was found to have a higher immunity level than the others and therefore selected for further studies. Restriction enzyme analysis showed that in plasmid pSS17-7 four copies of the *Sma*I–*Sal*I fragment is integrated into the *Hinc*II site of plasmid pACYC177 (data not shown).

CAT assay

The acetylation of [¹⁴C]chloramphenicol was analyzed by silica gel t.l.c. as described by Gorman *et al.*, (1982). After autoradiography the spots were cut out and counted. The protein concentration was determined by the method of Bradford (1976).

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