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The Bacteriophage 434 Right Operator

Roles of O_R1, O_R2 and O_R3

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Lysogenic induction of bacteriophage λ is controlled by the action of the phage repressor and Cro proteins at the phage right operator (O_R). This study examines the roles of the repressor and Cro proteins of the related phage 434. The start sites of transcription of the divergently oriented promoters in the 434 O_R region, P_R and P_{RM}, were mapped, and the effects of 434 repressor and Cro on promoter activity were assessed using promoter fusions to *lacZ*. The effects of repressor or Cro bound to each of the operator subsites (O_R1, O_R2 and O_R3) were assessed by examining regulation in the presence of operator mutations. The binding of Cro to a 434 operator was probed by an ethylation interference experiment which, together with other data, indicates that 434 Cro and repressor probably turn off transcription by blocking binding of RNA polymerase to promoter sequences. In general, the 434 and λ right operators are controlled in a similar fashion, but differences in detail were also encountered: (1) 434 Cro represses transcription from P_R primarily by binding to O_R1, whereas binding of λ Cro to O_R1 and O_R2 contribute equally to repression. (2) The 434 *cI* message, unlike that of λ, has a recognizable homology to the Shine–Dalgarno ribosome binding site. (3) Occupancy of O_R3 by repressor may be somewhat greater in a 434 lysogen than in a λ lysogen. (4) The 434 repressor probably activates transcription when bound at O_R2 by contacting RNA polymerase, as does λ repressor, but also by influencing competition between P_R and P_{RM}.

An analysis of the six right operator systems for which data are available indicates that all six repressors may employ the mechanism of transcriptional activation first described for λ, P22 and 434: apposition of an acidic surface to a particular part of RNA polymerase.

Keywords: phage 434; 434 right operator; 434 repressor; 434 Cro; phage induction

1. Introduction

Bacteriophages of the lambdoid family possess a regulatory region, the right operator (O_R), that is the locus of events controlling the choice between lysogeny and induction. Each right operator region contains three sites (O_R1, O_R2 and O_R3) that are specifically bound by the cognate phage repressor and Cro proteins, and two divergently oriented promoters (P_R and P_{RM}). P_R controls transcription of *cro* and lytic functions in the right operon, and P_{RM} controls transcription of *cI*, the gene for repressor. Phage induction is accompanied by a switch from leftward transcription of *cI* to rightward transcription of *cro* (for a review, see Ptashne, 1986).

Comparisons among the lambdoid phages have added to our understanding of O_R function by allowing conserved and presumably critical features to be distinguished from non-conserved idiosyncracies. Transcriptional control at O_R has been characterized in detail in the λ and P22 systems (see Ptashne, 1986 for references); studies of O_R regulation have also been reported for phages 434 (Lauer *et al.*, 1981; Wharton *et al.*, 1984; Bushman & Ptashne, 1986, 1988), Φ80 (Ogawa *et al.*, 1988*a*,*b*), and HK022 (Cam *et al.*, 1991). In addition, a right operator-like region has been characterized in the *Escherichia coli dicAC* region (Bejar *et al.*, 1988).

The repressor and Cro proteins of phage 434 have been subjected to extensive structural studies, but transcriptional control by these proteins has not been studied in depth; thus a detailed comparison of transcriptional control in 434 with that in λ and P22 seems particularly useful. In fact, the immunity regions of 434, λ, and P22 differ from one another in several respects. (1) The repressor and Cro proteins of each phage show only modest amino acid sequence similarity with the repressor and Cro pro-

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teins of the other phages. (2) The 434 operators are 14 bp† long, whereas the λ operators are 17 bp and the P22 operators are 18 bp, and the operators share little DNA sequence similarity between phages. (3) The relationship of the three operator sites to the promoters they control differs for each phage. (4) The repressor and Cro proteins of 434 and λ are all members of the helix-turn-helix family of DNA binding proteins, but each protein adopts a unique folded structure and each forms a distinctive complex with operator DNA (Aggarwal *et al.*, 1988; Anderson *et al.*, 1985; Brennan *et al.*, 1990; Jordan & Pabo, 1988; Wolberger *et al.*, 1988; for a recent review, see Harrison, 1991). The structures of P22 repressor and Cro have not been reported.

In this study, the sites of transcription initiation of 434 P_R and P_{RM} were determined, and promoter activities were assessed in the presence of repressor or Cro using gene fusions *in vivo*. In order to deduce the effects of repressor or Cro at specific sites, operator mutations were introduced into 434 O_R and the effects of these mutations on promoter activity were determined. In addition, interactions between 434 Cro and a 434 operator were characterized by an ethylation interference experiment. These data indicate that 434 repressor and Cro control transcription by acting at O_R1, O_R2 and O_R3 as in the λ paradigm, but these studies do reveal some differences in gene control in the two systems.

2. Materials and Methods

(a) *Bacteria and phages*

Strains and plasmids used in this study are described in Table 1. Phage stocks and lysogens were prepared, and β-galactosidase assays were performed according to Miller (1972). In β-galactosidase assays in which IPTG was used to control P_{lac}, cells were grown overnight in medium containing IPTG, and then diluted into fresh medium containing IPTG and grown to mid-log phase. Lysogens bearing *lacZ* fusions were judged to carry a single copy of the prophage by *ter* excision tests (Mousset & Thomas, 1969) or by comparing β-galactosidase levels in several isolates. F′ episomes were introduced into the appropriate US3 lysogens by selecting for streptomycin resistance and kanamycin resistance in spot matings with NK7047 (note that NK7047 is phenotypically *lacZ*$^-$ due to a transposon insertion). Additional methods were as described by Maniatis *et al.* (1982).

Operon fusions in which transcription of *lacZ* is controlled by 434 P_R or P_{RM} were constructed essentially as described (Meyer *et al.*, 1980; Bushman & Ptashne, 1988). In these fusions, 434 O_R and mutant derivatives were cloned into a λ phage carrying the *lacZ* coding region but not P_{lac} in a replaceable region of the λ genome. DNA fragments containing 434 O_R and mutant derivatives were isolated from the following plasmids: pRW81 (O_R^+), pRW82 (O_R3^-; from (λimm^{434}Tomizawa)), pRW83 (O_R1^-; from vir2), and pRW85 (O_R2^-; from vir15). DNA fragments containing O_R were isolated after cleavage of plasmid DNA with *Hin*dIII, and purified DNA fragments were ligated with *Hin*dIII-cleaved λ132 DNA. After packaging into λ phage heads *in vitro*, phage bearing fusions of 434 P_R to *lacZ* were identified as strains that formed red plaques on a lawn of US3 when plated on MacConkey lactose fermentation indicator plates but white plaques on a US3 derivative containing a λimm^{434} (Meselson) prophage. P_R-fusion phages λFB1, 3, 5 and 7 (bearing P_R in wild-type, O_R3^-, O_R1^- and O_R2^- backgrounds) were isolated in this manner. Phages bearing fusion of 434 P_{RM} O_R^+ to *lacZ* formed red plaques on a λimm^{434}(Meselson) lysogen and white plaques on US3 on MacConkey lactose plates. A $P_{RM}O_R^+$ *lacZ* fusion phage was isolated using this screen and named λFB2. A $P_{RM}O_R1^-$ *lacZ* fusion (named λFB6) was isolated by screening for light red plaques on US3 bearing pPN5-2, a plasmid that produces an amino-terminal fragment of 434 repressor (Anderson, 1984). (P_{RM} in an O_R1^- operator can be slightly stimulated by the amino-terminal domain of repressor (unpublished data), though it cannot be stimulated by intact repressor; Fig. 3(b)). A phage bearing a $P_{RM}O_R2^-$ fusion was identified by Benton-Davis plaque hybridization screening using the insert fragment as probe and named λFB8. A P_{RM} fusion bearing mutations in both O_R1 and O_R3 was also constructed. Plasmid pFB43 (Bushman & Ptashne, 1986) contains a deletion derivative of 434 O_R that removes O_R1 and inactivates P_R, and also a point mutation in O_R3 (that of λimm^{434}(Tomizawa)). To fuse P_{RM} to *lacZ*, the *Eco*RI to *Hin*dIII fragments bearing P_{RM} were excised and used as linkers between the left and right arms of 2 different phages. DNA from λ132*lacZ*$^-$h80 cleaved with *Hin*dIII was used to supply the right arm, and DNA from λ200$^{\lambda}$R1^0 cleaved at its single *Eco*RI site was used to supply the left arm. These DNAs were mixed with the P_{RM} containing linker fragments and ligated. After packaging *in vitro*, phage of the desired structure (named λFB9) were identified by 3 criteria: (1) ability to form plaques on a λ lysogen (excluding religated λ200i$^{\lambda}$R1^0), (2) *lacZ*$^+$ phenotype when plated in X-gal-containing agar on JM101 (excluding religated λ132*lacZ*$^-$h80), and (3) Benton-Davis hybridization screening using the 434 linker fragment as probe. DNA was isolated from all *lacZ* transducing phages, and the structure and orientation of the inserts were confirmed by restriction mapping.

(b) *S_1 nuclease protection*

S_1 nuclease protection assays were performed essentially as described (Brosius *et al.*, 1982). RNA transcripts were synthesized *in vitro* as described (Bushman & Ptashne, 1986) except that no labeled nucleotides were included in the reaction. The transcription templates used were the O_R-containing *Bam*HI to *Eco*RI DNA fragment from RP16 (Lauer *et al.*, 1981), for analyzing P_R, and the O_R-containing *Hin*dIII fragment from pFB20 (Bushman & Ptashne, 1986), for analyzing P_{RM}. RNA transcription products were treated with RNase-free DNase, phenol-extracted, and analyzed as described (Brosius *et al.*, 1982). Two DNA fragments from pRP16 were used as probes: (1) the *Bam*HI to *Alu*I fragment spanning 434 O_R 5′-labeled at the *Bam*HI site (P_R probe); and (2) the *Eco*RI to *Hin*dIII fragment spanning O_R 5′-labeled at the *Eco*RI site (P_{RM} probe).

(c) *5′-Nucleotide determination*

The 5′-triphosphate from each RNA transcript was isolated and characterized. Transcripts from each of the 2 promoters were labeled with 4[α-^{32}P]NTPs in separate transcription reactions (performed as described by

† Abbreviations used: bp, base-pair(s); IPTG, isopropyl-β,D-thiogalactopyranoside; X-gal, 5-bromo-4-chloro-3-indolyl-β,D-galactopyranoside.

Bushman & Ptashne, 1986), and then gel isolated. Each RNA sample was digested to monophosphates with nuclease P_1, and separated into monophosphate and triphosphate components by ascending chromatography on PEI-cellulose. After autoradiography, only 1 of the 4 RNA samples from each transcript showed a strong radioactive spot with the mobility of the triphosphate, thereby identifying the 5′-nucleotide of the RNA. For both P_R and P_{RM}, labeled triphosphate signals established that the prodominant 5′-nucleotide was ATP (data not shown). As a control, the known 5′-nucleotide of the λ P_R transcript was determined by this method and found as expected (Meyer, 1979) to be ATP.

Transcription from P_R and P_{RM} was inefficient in the presence of low concentrations of ATP compared with each of the other 3 NTPs in low concentration (data not shown). Since for many promoters, high concentrations of the initiating nucleotide are known to prime transcription, this observation is interpreted as a further indication that the 5′-nucleotides are predominantly ATP.

Dinucleotides corresponding to sequences around the RNA initiation site often stimulate transcription and so, as a further check on the location of the 5′-ends, the 16 dinucleotides were assayed for their ability to stimulate run-off transcription from 434 P_R and P_{RM}. For P_R, ApA, GpA and ApG stimulated transcription, while for P_{RM}, UpA and GpG stimulated transcription (data not shown). These observations indicate that the A residues within the brackets in Fig. 1 are the predominant points of transcription initiation; however, for the case of P_{RM}, the stimulation by GpG, together with the additional RNA 5′-ends identified by S_1, indicates that a fraction of the P_{RM} transcripts may start in the upstream sequence of 5 dG residues.

(d) *Ethylation interference*

Ethylation interference analysis of the interaction between 434 Cro and 434 operator was carried out as described (Bushman *et al.*, 1985), except that 434 Cro was substituted for 434 repressor.

3. Results

(a) *The promoters in 434 O_R*

S_1 nuclease mapping was used to identify the 5′-ends of RNAs initiating at 434 P_R and P_{RM} (Berk & Sharp, 1977). Previous work established that 434 repressor activates transcription of 434 P_{RM} and represses transcription from P_R *in vitro* (Bushman & Ptashne, 1986). RNA was synthesized *in vitro* from 434 O_R-containing DNA templates in the presence and absence of repressor, and samples were then annealed to 5′-end labeled DNA fragments spanning

Table 1

Bacterial strains, phages and plasmids used in this work

Strain or plasmid	Description or genotype	Source or reference
A. *Bacteria*		
US3	*RecA Strep^r F$^-$ lacZ ΔMM5265 his lacY$^+$*	M. Ptashne
NK7047F′	*lacI^{Q1} lacZ* :: Tn*5 (kan^r) proAB$^+$/Δ(lac proXIII) rpsE thi val^r*	N. Kleckner
JM101	*F′ traD36 lacIQ Δ(lacZ) M15 proAB/supE thi Δ(lac-proAB)*	Yanish-Perron *et al.* (1985)
B. *Phage*		
vir2	Virulent derivative of *434(λbio3h-1 O_R434 nin5)*	Wharton *et al.* (1984)
vir15	Virulent derivative of *434(λbio3h-1 O_R434 nin5)*	Wharton *et al.* (1984)
λimm^{434}(Meselson)	λ derivative bearing the immunity region of 434	M. Ptashne
λimm^{434}(Tomizawa)	Mutant derivative of λimm^{434}(Meselson)	J.-I. Tomizawa
λ132	i^{21} phage containing the *lacZ* coding region downstream of a unique *Hin*dIII site	Meyer *et al.* (1980)
λ132*lacZ*$^-$h80	*lacZ*-derivative of λ132 with h80 host range	M. Ptashne
λ200	i^{21} fusion of λP_R to *lacZ*	Meyer *et al.* (1980)
λ200i$^{\lambda}$R1^0	i$^{\lambda}$ derivative of λ200 with a single *Eco*RI site	M. Ptashne
λFB1	Carries 434 $P_RO_R^+$ fused to *lacZ*	This work
λFB2	Carries 434 $P_{RM}O_R^+$ fused to *lacZ*	This work
λFB3	Carries 434 $P_RO_R3^-$ fused to *lacZ*	This work
λFB4	Carries 434 $P_{RM}O_R3^-$ fused to *lacZ*	Bushman & Ptashne (1988)
λFB5	Carries 434 $P_RO_R1^-$ fused to *lacZ*	This work
λFB6	Carries 434 $P_{RM}O_R1^-$ fused to *lacZ*	This work
λFB7	Carries 434 $P_RO_R2^-$ fused to *lacZ*	This work
λFB8	Carries 434 $P_{RM}O_R2^-$ fused to *lacZ*	This work
λFB9	Carries 434 $P_{RM}\Delta$ (P_R, O_R1) O_R3^- fused to *lacZ*	This work
C. *Plasmids*		
pRW81	pBR322 derivative containing 434 O_R^+	Wharton (1985)
pRW82	Like pRW81, but with the O_R3^- mutation from λimm^{434}(Tomizawa)	Wharton (1985)
pRW83	Like pRW81, but with the O_R1^- from vir2	Wharton (1985)
pRW85	Like pRW81, but with the O_R2^- from vir15	Wharton (1985)
pPN5-2	pBR322 derivative expressing the amino-terminal domain of 434 repressor	Anderson (1984)
pFB20	pBR322 derivative containing 434 O_R O_R3^-	Bushman & Ptashne (1986)
pFB24	pBR322 derivative expressing 434 repressor under the control of a derivative of P_{lac}	Bushman & Ptashne (1988)
pFB43	pBR322 derivative containing 434 O_R Δ(P_R, O_R1) O_R3^-	Bushman & Ptashne (1986)
pGL139	pBR322 derivative expressing 434 Cro under control of P_{lac}	Lauer *et al.* (1981)
pRP16	pBR322 derivative expressing 434 repressor	Lauer *et al.* (1981)

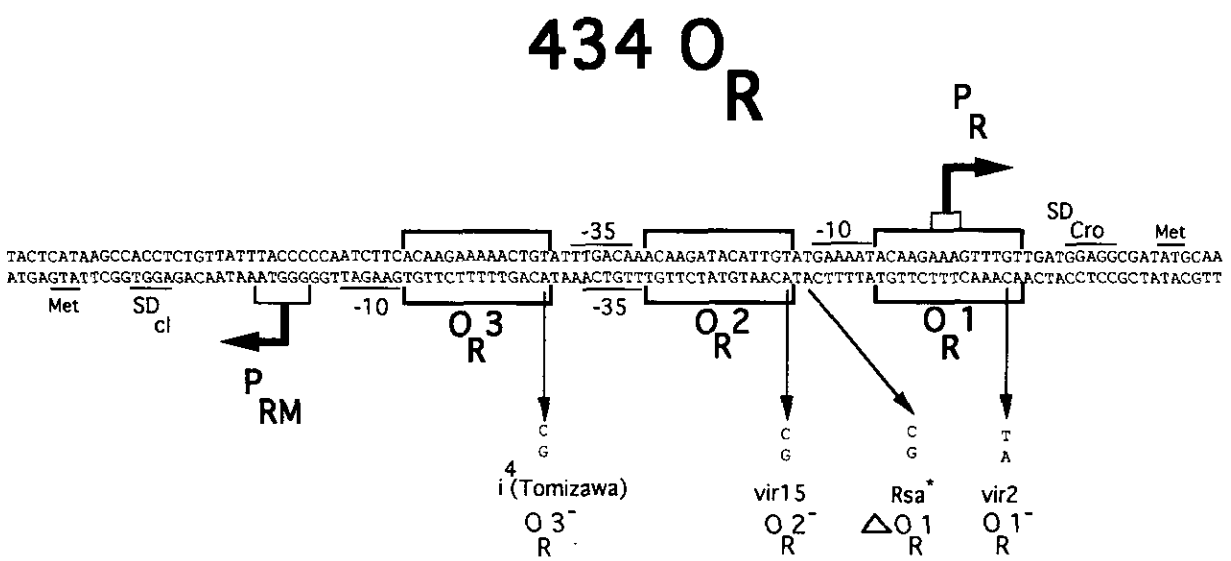

Figure 1. The 434 right operator. The 14 bp operators O_R1, O_R2 and O_R3 are enclosed in boxes, and the start-sites of transcription of the divergently oriented promoters P_R and P_{RM} are indicated with arrows. The 5′-ends identified by S_1 nuclease protection are marked by the brackets. The inferred −10 and −35 regions of P_R and P_{RM} are overlined and underlined, respectively. Candidate Shine–Dalgarno sequences are underlined and marked SD. The O_R1^- and O_R2^- mutants were isolated by Wharton (Wharton *et al.*, 1984), the λimm^{434}(Tomizawa) mutant was isolated by J.-I. Tomizawa and sequenced by Pirrotta (Pirrotta, 1979), and the *Rsa** mutant was introduced and used to delete O_R1 and P_R by digesting the mutant DNA with *Rsa*I as described (Bushman & Ptashne, 1986).

434 O_R. After digestion with S_1 nuclease, the sizes of protected DNA fragments were determined by electrophoresis adjacent to the products of DNA sequencing reactions (Maxam & Gilbert, 1977). The bottom strand probe protected RNAs that range in length from 43 to 50 bases. These RNAs are found only in reactions without repressor, and identify the start sites of the rightward promoter, P_R (Fig. 2). The top strand probe protected RNAs that range in length from 52 to 58 bases. These RNAs are found only in transcription reactions containing 434 repressor, and identify the start sites of transcription of the repressor-stimulated leftward promoter, P_{RM}. The points of transcription initiation are indicated with brackets in Figure 1. Isolation and characterization of the 5′-nucleotides of the P_R and P_{RM} messages, together with assays of transcriptional stimulation by dinucleotides, indicates that each message initiates primarily with A (data not shown). The location of the P_{RM} promoter is consistent with previous phosphate ethylation interference experiments (Bushman & Ptashne, 1986). Ovichinnikov *et al.* (1979) also determined the start site of transciption of 434 P_R and reached a similar conclusion.

The start site of transcription of P_R and P_{RM} lie downstream from reasonable matches to the −10 and −35 promoter sequence homologies (overlined and underlined in Fig. 1). P_R is a strong activator independent promoter and, as expected, matches the promoter consensus more closely than does P_{RM}.

(b) *Operator binding by 434 repressor and Cro*

In the following section, the effects of 434 repressor and Cro on transcription from 434 P_R and

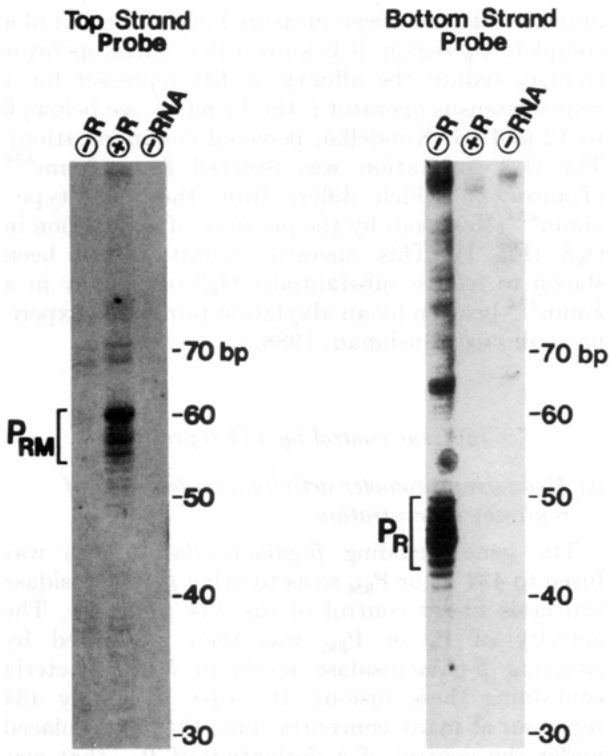

Figure 2. S_1 nuclease protection mapping of the promoters in 434 O_R. RNA synthesized *in vitro* in the presence (+R) or absence (−R) of 434 repressor was analyzed using the 5′-labeled DNA probes described in Materials and Methods. As a control, the experiment was repeated in the absence of added RNA. The sizes of protected DNA fragments were determined by co-electrophoresis with DNA sequencing ladders and end-labeled DNA fragments. Protected DNA fragments were aligned with the DNA sequence as described (Brosius *et al.*, 1982).

P_{RM} are interpreted in light of previous studies of the affinities of each protein for the three sites in O_R. Some important conclusions are as follows. Footprinting experiments *in vitro* have established that the repressor and Cro proteins of 434, like those of λ and P22, each bind to the three O_R sites with opposite orders of affinity: repressor binds most tightly to O_R1, whereas Cro binds most tightly to O_R3 (Wharton *et al.*, 1984). Repressor binds to each operator site as a dimer (Anderson *et al.*, 1985), and a repressor dimer bound to O_R1 can contact a second repressor dimer and help it bind to the adjacent lower affinity site O_R2. Thus, although O_R1 is the highest affinity site, O_R1 and O_R2 fill in the presence of the same concentration of repressor. Footprinting experiments conducted on a wild-type λimm^{434} lysogen have established that O_R1 and O_R2 are occupied by repressor *in vivo*, while O_R3 is partially occupied (Bushman, 1988). Co-operativity is pairwise: in an O_R1 mutant operator, O_R2 and O_R3 fill together (Wharton, 1985). Cro also binds DNA as a dimer (Wolberger *et al.*, 1988), but Cro dimers do not bind co-operatively (Wharton, 1985).

The mutations in O_R1 and O_R2 used in this study (Fig. 1) were initially isolated as *vir* mutations, and so are expected to decrease the affinity of 434 repressor for each mutant operator. Although the reduction in affinity resulting from these operator mutations has not been measured in the context of a complete O_R region, it is known that these operator changes reduce the affinity of 434 repressor for a near consensus operator ("the 14-mer", see below) 6 to 12-fold (G. Koudelka, personal communication). The O_R3^- mutation was isolated from λimm^{434} (Tomizawa), which differs from the "wild-type" λimm^{434}(Meselson) by the presence of a mutation in O_R3 (Fig. 1). This operator mutation has been shown to reduce substantially O_R3 occupancy in a λimm^{434} lysogen by an alkylation protection experiment *in vivo* (Bushman, 1988).

(c) *Gene control by 434 repressor*

(i) *Measuring promoter activity as a function of repressor concentration*

The gene encoding β-galactosidase (*lacZ*) was fused to 434 P_R or P_{RM} so as to bring β-galactosidase synthesis under control of the 434 promoter. The activity of P_R or P_{RM} was then monitored by assaying β-galactosidase levels in *lacZ*$^-$ bacteria containing these fusions. In order to supply 434 repressor at many concentrations, 434 *cI* was placed under the control of a derivative of P_{lac} that was carried on the plasmid pFB24. In strains containing pFB24 and a source *lac* repressor, the intracellular concentration of 434 repressor could be controlled by growing cells in the presence of different concentrations of the inducer IPTG (Maurer *et al.*, 1980; Meyer *et al.*, 1980). In this and the following sections, the genotypes of promoter fusions are denoted by first specifying the promoter controlling *lacZ* (P_R or P_{RM}) and the genotype of O_R.

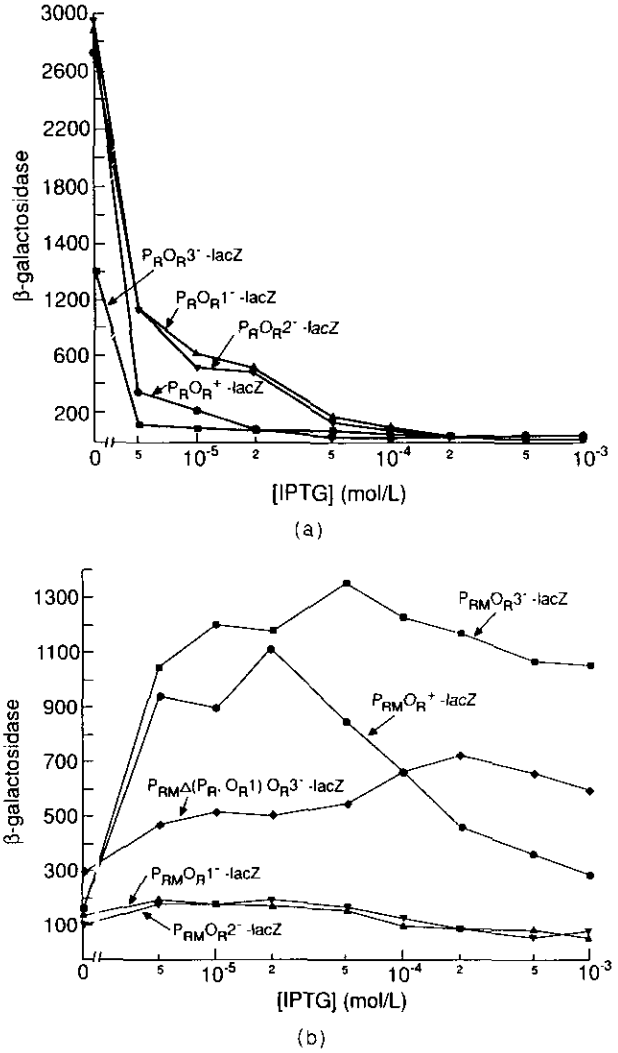

Figure 3. *The effects of mutations in O_R on turn-off of P_R and turn-on of P_{RM}.* (a) Levels of β-galactosidase measured in lysogens containing the indicated P_R-*lacZ* fusions and pFB24 in the presence of various concentrations of IPTG. pFB24 directs the synthesis of 434 repressor under the control of a derivative of P_{lac}. (b) Levels of β-galactosidase measured in lysogens containing the indicated P_{RM}-*lacZ* fusions and pFB24 in the presence of various concentrations of IPTG. The level of activity in the absence of 434 repressor was measured in the presence of vector plasmid pBR322.

(ii) *Turn-off P_R by repressor*

Figure 3(a) shows that $P_RO_R^+$ and $P_RO_R3^-$ were efficiently turned off by 434 repressor, while $P_RO_R1^-$ and $P_RO_R2^-$ were somewhat impaired for turn-off by repressor. In the presence of 2×10^{-5} M-IPTG, $P_RO_R^+$ was repressed 40-fold by repressor supplied from pFB24, while $P_RO_R1^-$ and $P_RO_R2^-$ were repressed less than ninefold. The observation that mutations in O_R1 and O_R2 diminish repression, while a mutation in O_R3 does not, supports the view that turn-off of P_R by repressor is effected by repressor at either O_R1 or O_R2.

The O_R1^- and O_R2^- point mutations used in these experiments (vir2 and vir15; see Fig. 1) lie outside the -10 and -35 conserved promoter sequences

and did not influence the activity of P_R. The shape of the curve describing repression of $P_RO_R3^-$ was similar to that for $P_RO_R^+$, indicating that $P_RO_R3^-$ was repressed as efficiently as $P_RO_R^+$, but unexpectedly the O_R3^- mutation used in these experiments reduced the activity of P_R about 2·5-fold. This point mutation lies at −38 with respect to the RNA start site, outside the TTGACA −35 consensus sequence. In one previous case, however, a base change at the −38 position (in λP_{RM}) was found to diminish promoter activity (Shih & Gussin, 1983).

(iii) *Activation of P_{RM} by repressor*

The roles of the operator sites in controlling P_{RM} were examined in the presence of many concentrations of repressor using the strategy described in the previous section (Fig. 3(b)). Comparison of curves describing repressor stimulation of $P_{RM}O_R^+$ and $P_{RM}O_R3^-$ revealed the role of O_R3. Both templates responded similarly to low concentrations of repressor, indicating that binding to O_R3 was not necessary for the six- to tenfold stimulation of P_{RM} observed in these experiments. At higher concentrations of IPTG, $P_{RM}O_R^+$ was repressed while $P_{RM}O_R3^-$ remained maximally active. These data indicate that O_R3 mediates negative control of P_{RM} at high concentrations of repressor; studies of double lysogens described in Table 2 (see below) also support this conclusion.

The $P_{RM}O_R2^-$ curve indicated that repressor bound to O_R2 is necessary for efficient activation of P_{RM}. When O_R2 was mutant, P_{RM} was stimulated no more than twofold.

Figure 3(b) shows that $P_{RM}O_R1^-$ was not stimulated by repressor, probably because repressor bound co-operatively to O_R2 and O_R3 on this template (Wharton, 1985). Thus repressor bound to O_R3 can block activation by repressor at O_R2 and so turn off P_{RM}. This explanation predicts that addition of an O_R3^- mutation to a $P_{RM}O_R1$ fusion should allow repressor to stimulate P_{RM}. Such a mutant was made by deleting DNA containing O_R1 from an O_R3 operator, and as expected stimulation of P_{RM} by repressor was observed in the $P_{RM}\Delta(P_R, O_R1)O_R3^-$ operator. The level of stimulation was not as high as with wild-type O_R, possibly because repressor at O_R2 promoted earlier filling of the mutant O_R3 due to pairwise co-operativity.

Table 2

Effects of 434 repressor supplied from λimm^{434}(Meselson) and λimm^{434}(Tomizawa) on P_R and P_{RM}

	Promoter/operator		
λimm^{434} prophage	$P_RO_R^+$	$P_{RM}O_R^+$	$P_{RM}O_R3^-$
None	2870	170	210
λimm^{434}(Meselson)	ND	1070	1540
λimm^{434}(Tomizawa)	20	860	1720

β-Galactosidase activity was measured in lysogens containing λFB1, λFB2 and λFB4 and the indicated 434 prophage. ND, not determined.

The deletion of P_R also resulted in a twofold increase in the basal level of transcription from P_{RM}. This result is as expected from a model in which RNA polymerase at P_R interferes with the activity of P_{RM}. Several other lines of evidence are also consistent with the view that P_R and P_{RM} compete, and that activation of P_{RM} is effected in part by repression of P_R (see Discussion).

(iv) *Promoter activity in λimm^{434} lysogens*

To determine the effects of lysogenic amounts of 434 repressor on the 434 promoters, λimm^{434} (Meselson) and λimm^{434}(Tomizawa) were introduced into bacterial strains containing $P_{RM}O_R^+$ or $P_{RM}O_R3^-$ fusions. Lysogens of λimm^{434}(Tomizawa) have been shown previously to contain more 434 repressor than lysogens of λimm^{434}(Meselson) (Pirrotta & Ptashne, 1969). Table 2 shows that repressor synthesized by either λimm^{434} prophage stimulated transcription from P_{RM}. More β-galactosidase activity was detected in strains containing the O_R3^- mutant fusion, suggesting that O_R3 is partially occupied by repressor in a lysogen and that the O_R3^- mutant relieves this repression.

(d) *Gene control by 434 Cro*

(i) *Turn off of P_R by Cro*

In the experiment shown in Figure 4(a), 434 Cro was supplied under control of P_{lac} from plasmid pGL139 (Lauer *et al.*, 1981); the regulatory effects of different concentrations of 434 Cro were assessed by varying the concentration of IPTG as in the experiments with repressor. The $P_RO_R^+$ curve shows that over the range of Cro concentrations used in this experiment, P_R was repressed sevenfold. Repression of $P_RO_R2^-$ by Cro was indistinguishable from $P_RO_R^+$, while repression of $P_RO_R1^-$ was significantly impaired. At 5×10^{-4} M-IPTG, $P_RO_R^+$ and $P_RO_R2^-$ were repressed fivefold, while $P_RO_R1^-$ was repressed only twofold. This result was as predicted from the affinities of Cro for sites in O_R: since Cro binds twofold more tightly to O_R1 than to O_R2 (Wharton *et al.*, 1984), O_R1^- mutant P_R derivatives were expected to require higher concentrations of Cro than O_R2^- derivatives to achieve a given degree of repression. $P_RO_R3^-$ was repressed by Cro as efficiently as $P_RO_R^+$, indicating that O_R3 has no role in repression of P_R by Cro.

(ii) *Control of P_{RM} by Cro*

Figure 4(b) presents an analysis of the roles of the operator sites in the regulation of P_{RM} by Cro. A $P_{RM}O_R^+$ fusion was repressed somewhat by Cro, with half-maximal repression occurring at between 2×10^{-5} and 5×10^{-5} M-IPTG; curves describing repression of $P_{RM}O_R1^-$ and $P_{RM}O_R2^-$ were indistinguishable from $P_{RM}O_R^+$, indicating that O_R1 and O_R2 play no role in the repression of P_{RM} by Cro.

An O_R3^- mutation not only relieved repression of P_{RM} by Cro, suggesting that turn off of P_{RM} is effected by Cro at O_R3, but also allowed Cro to

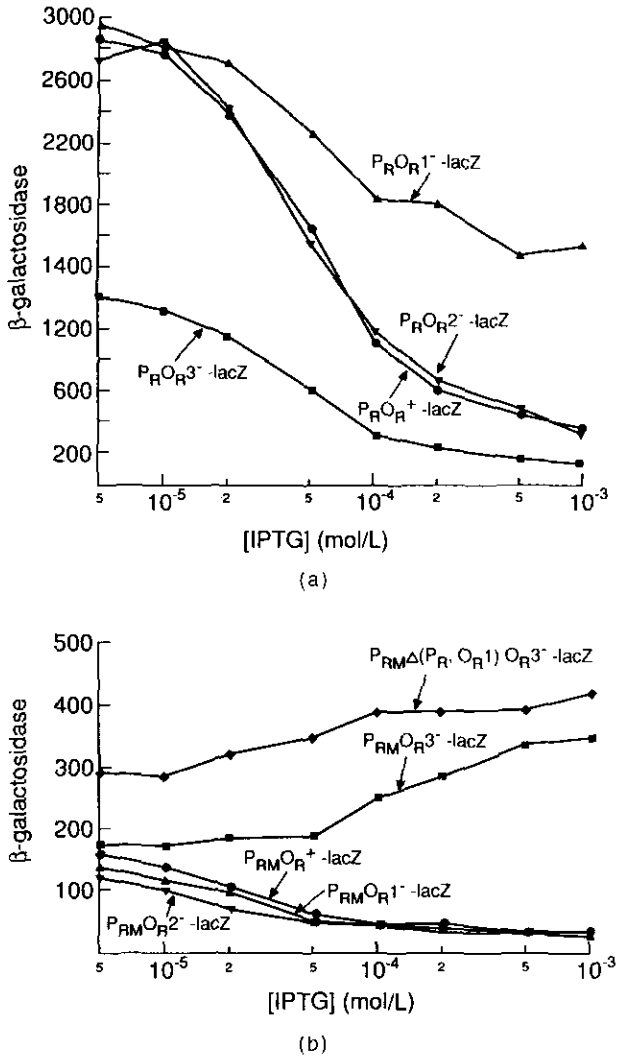

Figure 4. The effect of mutations in O_R on turn-off of P_R and turn-off of P_{RM} by 434 Cro. (a) Levels of β-galactosidase measured in lysogens containing the indicated P_R-*lacZ* fusions in the presence of pGL139 and various concentrations of IPTG. pGL139 directs the synthesis of 434 Cro under the control of P_{lac}. (b) Levels of β-galactosidase measured in lysogens of the indicated P_{RM}-*lacZ* fusions in the presence of pGL139 and various concentrations of IPTG.

stimulate P_{RM} about twofold. This effect might have been due to Cro excluding a competing RNA polymerase molecule from P_R, thereby allowing RNA polymerase access to P_{RM}. Alternatively, Cro might have contacted polymerase at P_{RM} directly and stimulated transcription through a protein–protein contact. To distinguish between these possibilities, stimulation of $P_{RM}\Delta(P_R, O_R1)O_R3^-$ by Cro was tested. It was expected that if Cro stimulated P_{RM} by excluding polymerase from P_R, inactivating P_R should reduce stimulation of P_{RM} by Cro. Figure 4(b) shows that stimulation of $P_{RM}\Delta(P_R, O_R1)O_R3^-$ was slightly reduced compared with $P_{RM}O_R3^-$. These results, though modest quantitatively, support the idea that Cro at O_R2 turned on P_{RM} at least in part by excluding competing polymerase from P_R.

(iii) *Cro antagonizes stimulation of P_{RM} by repressor*

Upon treatment of a lysogen with an inducing signal, RecA protein is converted to a form that promotes cleavage of phage repressors. The concentration of active repressor thus falls, allowing some Cro protein to accumulate. Cro can then bind to O_R3, turning off further synthesis of repressor and committing a lysogen to induction and lytic growth (Ptashne, 1986).

The results presented in Table 3 test the expectation that 434 Cro bound to O_R3 antagonizes stimulation of P_{RM} by 434 repressor at O_R2. Strains containing the indicated P_{RM} fusion were made lysogenic for λimm^{434}(Tomizawa), and then transformed with either pGL139, which directs the synthesis of 434 Cro, or with pBR322 as a control. In the absence of Cro, 434 repressor from the λimm^{434} prophage stimulated P_{RM}. In the presence of Cro, the activity of $P_{RM}O_R^+$ was reduced tenfold, while the activity of $P_{RM}O_R3^-$ was reduced only 20%. Thus 434 Cro, like λ Cro, could block transcription from P_{RM} even when P_{RM} was stimulated by repressor.

(e) *Interactions between 434 Cro and the operator phosphate backbone*

On the basis of prior experiments in other phage systems, one would expect that 434 repressor and Cro would turn off transcription by blocking access of RNA polymerase to promoter sequences. Previous mapping of the binding sites of proteins in phage right operator regions has helped provide evidence for this mechanism (for a review, see Ptashne, 1986). To gain insight into the mechanism of negative control by 434 Cro, the positions of close approach between 434 Cro and the operator phosphate backbone were determined in an ethylation interference experiment.

DNA molecules containing a 434 operator were end-labeled and ethylated so that each molecule contained on average one ethylphosphate. The ethylated DNA molecules were incubated with excess 434 Cro, and separated into Cro-bound and free fractions by gel electrophoresis. DNA molecules in the free fraction contained ethylations that interfere with binding of Cro to the operator. Bound and free

Table 3
434 Cro antagonizes stimulation of P_{RM} by 434 repressor

Plasmid	*Promoter/operator fused to lacZ* relative β-galactosidase activity $P_{RM}O_R^+$	$P_{RM}O_R3^-$
pBR322 (vector)	1·0	1·0
pGL139 (434 Cro)	0·1	0·8

Strains contained 2 prophages, λimm^{434}(Tomizawa) and the indicated *lacZ* fusion phage (λFB2 or λFB4). Maximum levels of activity (relative activity of 1·0) are as in Table 2. The presence of 434 Cro does not induce the λimm^{434}(Tomizawa) lysogen because of the presence of the mutation in O_R3.

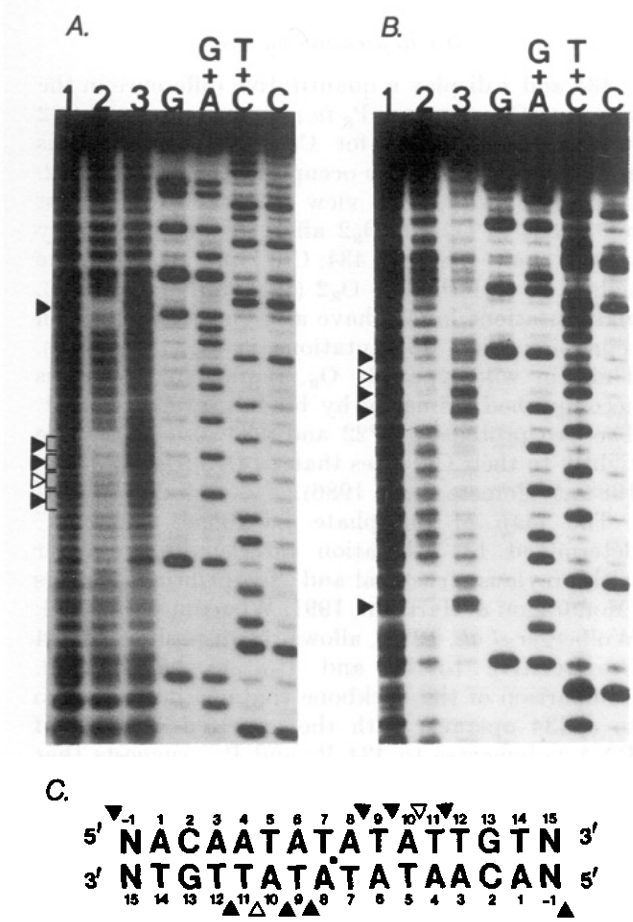

Figure 5. Ethylation interference analysis of interactions between 434 Cro and a 434 operator. A, Results for the top strand. B, Results for the bottom strand. Lanes: 1, ethylated DNA cleaved at the positions of ethylphosphates; 2, DNA bound by Cro cleaved at the positions of ethylphosphate groups; 3, DNA not bound by Cro cleaved at the positions of ethylphosphate groups. In A and B, bands depleted in lane 2 and enriched in lane 3 relative to the control in lane 1 indicate ethylphosphate groups that interfere with binding of Cro. Filled triangles, strong interferences; open triangles, weaker interference. Bound and free DNA fractions were separated in a standard "band shift" gel as described except that 9×10^{-8} M-Cro was substituted for 2×10^{-7} M-repressor. The 434 operator DNA site used in the experiment is the near-consensus "14-mer" (Anderson *et al.*, 1984). The products of cleavage at ethylphosphate groups were aligned with the control Maxam–Gilbert sequencing ladder as described (Siebenlist & Gilbert, 1980). C, Ethylphosphate groups that interfere with binding of Cro aligned with the 434 operator sequence.

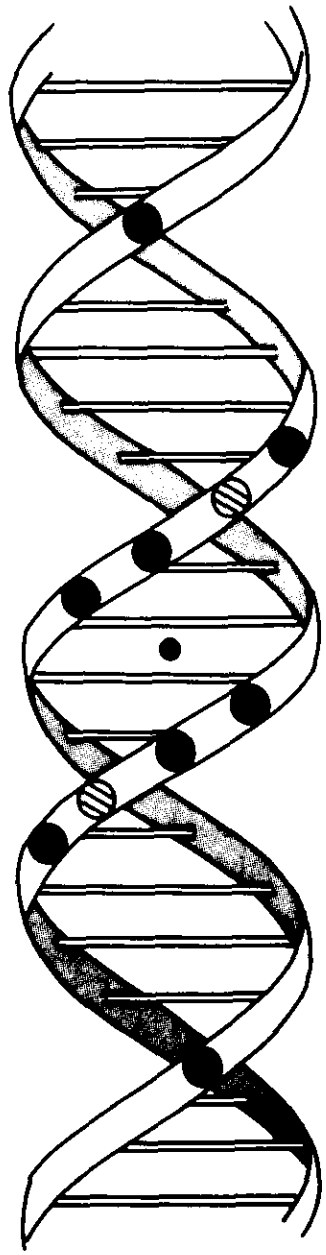

Figure 6. Phosphate groups of the 434 operator at which ethylation reduces the affinity of 434 Cro displayed on a DNA duplex. Positions of strong ethylation interference are indicated by filled circles and weaker interferences by hatched circles on the *B*-DNA helix. The operator center of symmetry is marked with a small filled circle.

fractions were recovered from the gel, cleaved at the positions of ethylphosphate groups by heating in base, and examined by electrophoresis on DNA sequencing gels. Separate experiments were performed for each of the DNA strands (Fig. 5A and B). Each band on the gel corresponds to cleavage at a specific ethylphosphate group. Bands enriched in the free fraction and depleted in the bound fraction identified phosphate groups where ethylation interferes with binding of Cro. The positions of ethylation interference were symmetrically disposed around the center of the operator (Fig. 5C), and lay on one face of the DNA helix (Fig. 6).

4. Discussion

Despite the differences between the immunity regions of 434 and the other lambdoid phages, the control of transcription in 434 generally follows the rules first worked out in the λ and P22 systems (Ptashne, 1986). Specifically, 434 repressor or Cro

bound to O_R1 or O_R2 turns off transcription from P_R. Repressor or Cro bound to O_R3 turns off P_{RM}, and Cro bound to O_R3 turns off P_{RM} even in the presence of repressor at O_R2. Cro, unlike repressor, does not stimulate transcription efficiently when bound to O_R2. Previous studies of gene control by 434 repressor *in vitro* also support some of these points (Bushman & Ptashne, 1986, 1988). Features of gene control by phage 434 that depart from the λ paradigm are discussed below.

(a) *Occupancy of O_R3 in a 434 lysogen*

The phenotypic differences between lysogens of the λimm^{434}(Meselson) and λimm^{434}(Tomizawa) strains of λimm^{434} are probably explained by relief of autogenous negative control by repressor at O_R3 and a consequent increase in repressor levels. Stocks of either λimm^{434} phage contain frequent mutants that are able to grow on λimm^{434}(Meselson) but not λimm^{434}(Tomizawa) lysogens (Ptashne, 1971). Pirrotta & Ptashne (1969) found that λimm^{434} (Tomizawa) lysogens contain more 434 repressor than do λimm^{434} (Meselson) lysogens, and suggested that the higher repressor levels in λimm^{434} (Tomizawa) lysogens may render them immune to the "single step" virulent mutants that grow on λimm^{434}(Meselson) lysogens. These workers suggested that λimm^{434} (Tomizawa) might contain a mutation in the promoter directing repressor synthesis that increased its activity (Ptashne, 1971), or a mutation in O_R3 that diminishes autogenous negative control (Pirrotta, 1979), but were unable to distinguish between these alternatives. Results presented here indicate that the mutation in λimm^{434}(Tomizawa) lies outside the P_{RM} consensus sequences, and does not substantially affect P_{RM} activity in promoter fusions. Repression at O_R3, however, is drastically reduced (Table 2 and Fig. 3(b)), arguing that the higher repressor levels in λimm^{434}(Tomizawa) are due to relief of repression at O_R3.

434 and λ may differ in the degree of occupancy of O_R3 in a lysogen. In the λ case, experiments with gene fusions *in vivo* suggest that O_R3 is less than 20% occupied (Maurer *et al.*, 1980). In 434, comparison of β-galactosidase measurements of $P_{RM}O_R^+$-*lacZ* and $P_{RM}O_R3^-$-*lacZ* in the presence of lysogenic amounts of repressor suggests that λimm^{434}(Meselson) produces enough repressor for 30% occupancy of O_R3 and λimm^{434}(Tomizawa) produces enough repressor for 50% occupancy of O_R3 (Table 2). A dimethyl sulfate protection experiment *in vivo* reveals roughly 50% protection of O_R3 in an λimm^{434}(Meselson) lysogen but no obvious protection of O_R3 in an λimm^{434}(Tomizawa) lysogen (Bushman, 1988). Comparison of lysogenic levels of repressor in λ and 434 suggests that the λimm^{434} (Tomizawa) level is quite close to that of λ, while the λimm^{434} (Meselson) level is five times lower (Levine *et al.*, 1979). The lower level of repressor in λimm^{434}(Meselson) lysogens compared with λ lysogens is thus probably also due to higher occupancy of O_R3.

(b) *Repression by Cro*

434 and λ display a quantitative difference in the way that Cro represses P_R *in vivo*. In λ, O_R1 and O_R2 have equal affinities for Cro, and repression is accomplished when Cro occupies either O_R1 or O_R2. Consistent with this view is the finding that mutations in O_R1 or O_R2 affect repression equally (Meyer *et al.*, 1980). In 434, Cro binds twofold more tightly to O_R1 than to O_R2 (Wharton *et al.*, 1984), and mutations in O_R1 have a more severe effect on repression than do mutations in O_R2 (Fig. 4(a)). Thus, in wild-type 434 O_R, repression by Cro is accomplished primarily by binding of Cro to O_R1. The Cro proteins of P22 and Φ80 also bind more tightly to their O_R1 sites than to O_R2 (Ogawa *et al.*, 1988*a*,*b*; Poteete *et al.*, 1986).

The map of phosphate backbone "contacts" determined by ethylation interference, together with previous structural and "footprinting" studies (Mondragon & Harrison, 1991; Wharton *et al.*, 1984; Wolberger *et al.*, 1988), allows the disposition of 434 Cro relative to P_R and P_{RM} to be assessed. Comparison of the backbone contacts made by Cro to a 434 operator with the expected contacts of RNA polymerase to 434 P_R and P_{RM} suggests that 434 Cro bound to O_R3 would contact some of the same phosphate groups as RNA polymerase bound to P_{RM}. At O_R2, Cro contacts several phosphate groups that are expected to be contacted by RNA polymerase at P_R; at O_R1, Cro would cover the start site of transcription. Thus, it seems likely that Cro turns off transcription by blocking the access of RNA polymerase to the 434 promoter sequences. A similar analysis of contacts made by 434 repressor (Bushman *et al.*, 1985) also suggests that repressor turns off transcription by blocking binding of RNA polymerase.

(c) *Translation of* cI

The identification of the 434 P_{RM} transcription start site establishes that the AUG translation start codon of 434 *cI*, unlike that of λ, is preceded by a reasonable homology to the Shine–Dalgarno ribosome binding sequence (Fig. 1). The phage Φ80 *cI* message also contains a Shine–Dalgarno sequence (Ogawa *et al.*, 1988*a*,*b*), while the λ and HK022 *cI* messages, in contrast, each begin with the AUG itself (Meyer, 1979; Cam *et al.*, 1991). The *cI* message of P22, and probably also the *cI* message of the related *DicAC* region, both initiate only three bases upstream from the start of the coding region and lack Shine–Dalgarno sequences (Poteete & Ptashne, 1982; Bejar *et al.*, 1988). The λ and HK022 *cI* messages contain a region just downstream from the AUG that is required for efficient translation (Sheen & Gottesman, 1992). Each *cI* message that lacks a Shine–Dalgarno sequence contains a reasonable match to the "downstream" box, while 434 and Φ80, which contain Shine–Dalgarno sequences, have weaker matches to the downstream box. The data taken together suggest that each *cI* message

requires a sequence to promote efficient translation, and this requirement can be met either by a downstream box or a Shine–Dalgarno sequence.

(d) *Mechanism of activation*

(i) *Promoter competition*

It has been proposed that the repressors of phages lambda, 434 and P22 stimulate transcription of their cognate P_{RM} promoters by directly contacting RNA polymerase (Guarente *et al.*, 1982; Hochschild *et al.*, 1983; Bushman & Ptashne, 1986, 1988; Bushman *et al.*, 1989); data presented here suggest that a second mechanism of activation may also operate in the 434 case. Binding of RNA polymerase to 434 P_R may interfere with binding of polymerase to P_{RM}, and transcription from P_{RM} may be stimulated slightly by blocking the binding of polymerase to P_R. Such a model has been proposed previously to explain the activation of P22 P_{mnt} (Vershon *et al.*, 1987) and several other prokaryotic promoters (Goodrich & McClure, 1991). The mapping of the 434 P_R and P_{RM} promoters reveals that the −35 regions of the two promoters actually overlap (Fig. 1), supporting the idea that polymerase bound to one promoter might interfere with binding to the other. Four experimental findings are consistent with a competition model. (1) Deleting P_R raises the basal level of P_{RM} about twofold (Fig. 3(b)). (2) Cro activates transcription twofold on templates bearing a mutation in O_R3 that eliminates turn-off of P_{RM} by Cro, but when P_R is inactivated, this stimulation by Cro is reduced (Fig. 4(b)). (3) Repressor can activate transcription 50% to twofold on templates mutant in O_R2 (Fig. 3(b) and Bushman & Ptashne, 1986) (an alternative interpretation of this observation is also possible: repressor may partially fill O_R2 by virtue of cooperativity with repressor at O_R1). (4) Unlike the λ case, mutant derivatives of 434 repressor containing alterations in the region proposed to contact RNA polymerase only partially obstruct activation (Bushman & Ptashne, 1988).

(ii) *Conserved aspects of transcriptional activation*

Chemically similar interactions between repressor at O_R2 and RNA polymerase at P_{RM} probably mediate activation in all six right operator systems for which data are available (434, λ, P22, Φ80, HK022, and the chromosomal Dic operon). Each of the repressors are known or inferred to be members of the helix-turn-helix family of DNA-binding proteins. Acidic residues in the helix-turn-helix unit have been shown previously to be important for transcriptional activation in studies of 434, λ, P22. In Φ80, HK022, and DicA also, each repressor bound at O_R2 is positioned to appose an acidic part of its helix-turn-helix unit to a part of RNA polymerase implicated previously in activation. The evidence for this view is outlined in more detail below.

Mutants of λ repressor and P22 repressor were isolated that were specifically impaired in activation of P_{RM}. The changes in these pc (*positive control*) mutants were found to have a common chemical character: each made repressor more basic. All such mutants isolated in λ repressor were found to cluster on the surface of helix 2 and the loop between helices 2 and 3. A pseudo-revertant of a λ pc mutation that increased activation of P_{RM} substituted an acidic residue for an unchanged residue in this region (Guarante *et al.*, 1982; Hochschild *et al.*, 1983). The importance of acidic residues in transcriptional activation was further demonstrated by converting λ Cro into a transcriptional activator by altering it to bear an acidic surface as in repressor; when the modified Cro was bound at O_R2 it too stimulated transcription from P_{RM} (Bushman *et al.*, 1988). In P22, a single positive control mutation was isolated that changed an acidic residue to a basic residue in an inferred loop carboxyl-terminal of helix 3 (Hochschild *et al.*, 1983).

Both λ and P22 repressors apparently appose the acidic activating surface to the same part of RNA polymerase, but different parts of repressor are involved in each case. When the relative position of repressor at O_R2 and RNA polymerase at P_{RM} were compared in λ and P22 by analyzing contacts to the DNA phosphate backbone, it was found that contacts made by each repressor overlapped the contacts made by RNA polymerase at a single phosphate group. This "shared phosphate" was at the same point in each case with respect to the P_{RM} sequence (near the −35 region), but at a different point with respect to each O_R2 operator. Strikingly, the changes in the positive control mutants, clustered in the part of repressor expected to lie nearest the shared phosphate when repressor is bound at O_R2, even though in the two cases the substitutions lay on different surfaces of the repressor protein. On the basis of these observations it was proposed that the λ the P22 repressors activate transcription by apposing an acidic part of the protein surface to a single key part of RNA polymerase at the shared phosphate (Hochschild *et al.*, 1983).

In the case of 434, the determination of the start-site of transcription of 434 P_{RM} presented here, together with other data (Bushman & Ptashne, 1986, 1988), indicate that 434 repressor at 434 O_R2 closely approaches the key part of RNA polymerase using the solvent-exposed surface of helix 2, as does λ repressor. Mutations changing the charge of helix 2 of 434 repressor altered activation in the predicted way, increased negative charge increasing activation and decreased negative charge diminishing activation (Bushman & Ptashne, 1988), suggesting that 434 repressor also stimulates transcription by apposing an acidic surface to a single part of RNA polymerase.

Do the Φ80, HK022, and DicA repressors also contain acidic surfaces that can contact RNA polymerase as in λ, 434, and P22? Figure 7 presents the disposition of each of the six P_{RM} promoters relative to its O_R2 operator, which determines the part of repressor that most closely approaches the critical part of RNA polymerase. It can be seen that the

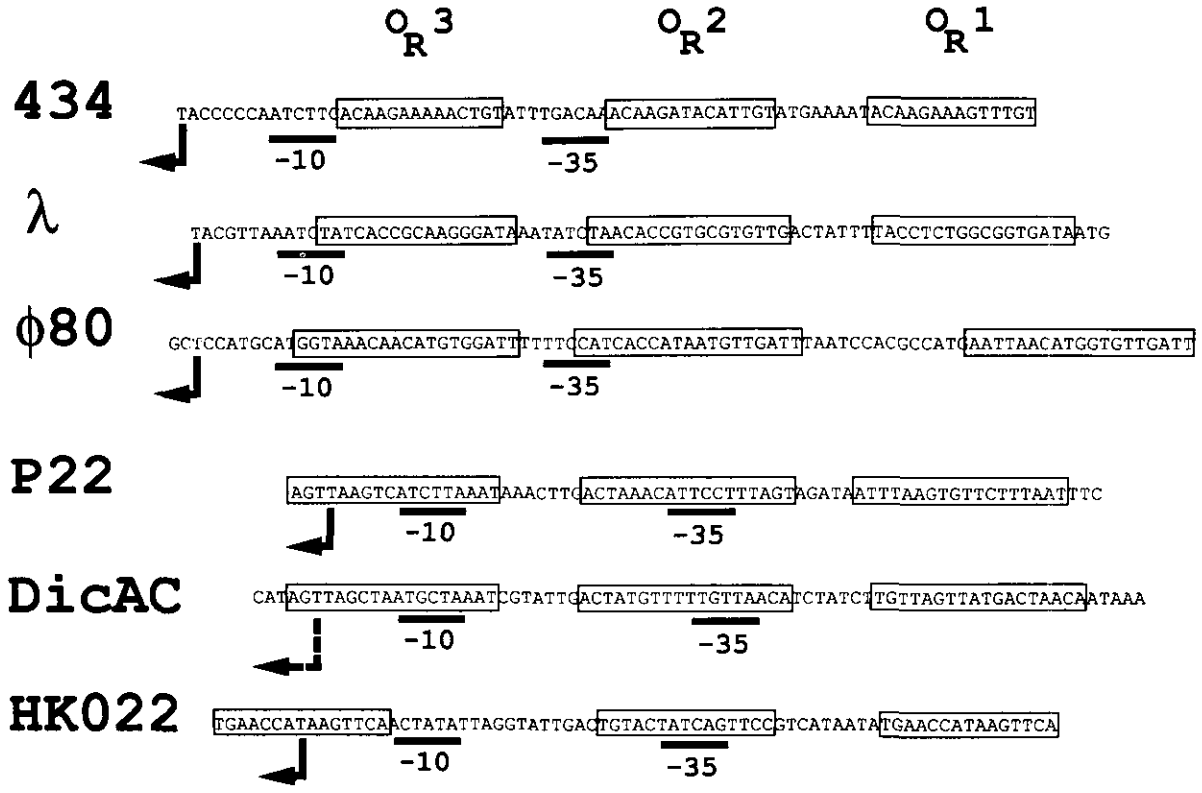

Figure 7. Disposition of P_{RM} promoters relative to O_R2. The start site of P_{RM} transcription is marked with an arrow; the operator sequences are enclosed in boxes. The −10 and −35 sequences of P_{RM} are underlined. References for the transcription start sites are as follows. 434, Bushman & Ptashne (1986) and this work; λ, Meyer (1979); Φ80, Ogawa *et al.* (1988*a*,*b*); P22, *Poteete & Ptashne (1982); HK022, Cam et al. (1991). The start-site of the* P_{RM} *analog of DicAC has not* been reported, but can be tentatively identified by comparison to the closely related phage P22 (Bejar *et al.*, 1988). For this reason the DicAC start-site is marked with the broken arrow.

alignments fall into two groups: one containing λ, 434, and Φ80, and the other containing P22, HK022, and DicA. Figure 8 shows the sequences of each repressor in the region of the helix-turn-helix, and the locations of the amino acid substitutions in the positive control mutants. Φ80 repressor, which has a λ-like alignment, has a net negative charge on the surface of helix 2, as do λ and 434 repressors; repressors *in the* P22 group do not. HK022 and DicA repressors, which have P22-like alignments, each have an acidic residue at the site of the P22 pc substitution, but repressors of the λ-434 group do not have acidic residues at this position. These observations indicate that the mechanism of positive control employed by the λ, P22 and 434 repressors, apposition of an acidic surface to a particular part of RNA polymerase, may also be employed by the Φ80, HK022, and DicA repressors.

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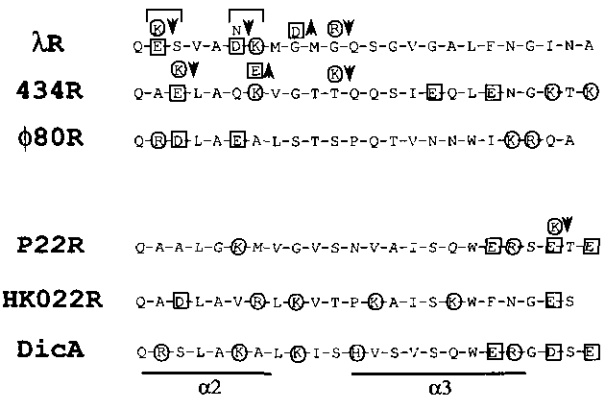

Figure 8. Sequences of the helix-turn-helix regions of several repressors, encompassing the probable transcriptional activating regions. The α helices of the helix-turn-helix are underlined. Acidic residues are enclosed in boxes, basic residues are enclosed in ovals. The sequences of the positive control mutants of Guarente *et al.* (1982), Hochschild *et al.* (1983) and Bushman & Ptashne (1988) are shown above the sequences. Mutants that diminish activation are marked with downward pointing arrows, those that improve activation are shown with upward pointing arrows. The residues found to be most important for activation by λ repressor by Bushman *et al.* (1989) are enclosed in brackets. References for additional helix-turn-helix sequences are as follows. Φ80, Ogawa *et al.* (1988*a*,*b*); HK022, Cam *et al.* (1991); DicA, Bejar *et al.* (1988).

References

Aggarwal, A. K., Rogers, D. W., Drottar, M., Ptashne, M. & Harrison, S. C. (1988). Recognition of a DNA operator by the repressor of phage 434: a view at high resolution. *Science*, **242**, 899–907.

Anderson, J. E. (1984). The 7 Å structure of a 434 repressor-operator complex. Dissertation, Harvard University, Cambridge, MA.

Anderson, J. E., Ptashne, M. & Harrison, S. C. (1984). Cocrystals of the DNA-binding domain of phage 434 repressor and a synthetic phage 434 operator. *Proc. Nat. Acad. Sci., U.S.A.* **81**, 1307–1311.

Anderson, J. E., Ptashne, M. & Harrison, S. C. (1985). The structure of a phage repressor-operator complex at 7 Å resolution. *Nature (London)*, **319**, 596–601.

Bejar, S., Bouche, F. & Bouche, J.-P. (1988). Cell division inhibition gene dicB is regulated by a locus similar to lambdoid bacteriophage immunity loci. *Mol. Gen. Genet.* **212**, 11–19.

Berk, A. J. & Sharpe, P. A. (1977). Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S_1 endonuclease digested hybrids. *Cell*, **12**, 721–732.

Brennan, R. G., Roderick, S. L., Takeda, Y. & Matthews, B. W. (1990). Protein-DNA conformational changes in the crystal structure of a λ Cro-operator complex. *Proc. Nat. Acad. Sci., U.S.A.* **87**, 8165 8169.

Brosius, J., Cate, R. L. & Perlmutter, A. P. (1982). Precise location of two promoters for the β-lactamase gene of pBR322. *J. Biol. Chem.* **257**, 9205–9210.

Bushman, F. D. (1988). Activation of transcription by bacteriophage repressors. Dissertation, Harvard University, Cambridge, MA.

Bushman, F. D. & Ptashne, M. (1986). Activation of transcription by the bacteriophage 434 repressor. *Proc. Nat. Acad. Sci., U.S.A.* **83**, 9353–9357.

Bushman, F. D. & Ptashne, M. (1988). Turning λ cro into a transcriptional activator. *Cell*, **54**, 191–197.

Bushman, F. D., Anderson, J. E., Harrison, S. C. & Ptashne, M. (1985). Ethylation interference and X-ray crystallography identify similar interactions between 434 repressor and operator. *Nature (London)*, **316**, 651–653.

Bushman, F. D., Shang, C. & Ptashne, M. (1989). A single glutamic acid residue plays a key role in the transcriptional activation function of λ repressor. *Cell*, **58**, 1163–1171.

Cam, K., Oberto, J. & Weisberg, R. A. (1991). The early promoters of bacteriophage HK022: contrasts and similarities to other lambdoid phages. *J. Bacteriol.* **173**, 734–740.

Goodrich, J. A. & McClure, W. R. (1991). Competing promoters in prokaryotic transcription. *Trends Biochem. Sci.* **16**, 394–397.

Guarente, L., Nye, J. S., Hochschild, A. & Ptashne, M. (1982). Mutant λ phage repressor with a specific defect in its positive control function. *Proc. Nat. Acad. Sci., U.S.A.* **79**, 2236–2239.

Harrison, S. C. (1991). A structural taxonomy of DNA-binding domains. *Nature (London)*, **353**, 715–719.

Hochschild, A., Irwin, N. & Ptashne, M. (1983). Repressor structure and the mechanism of positive control. *Cell*, **32**, 319–325.

Jordan, S. & Pabo, C. O. (1988). Structure of the λ complex at 2·5 Å resolution: details of the repressor-operator interactions. *Science*, **242**, 895 899.

Lauer, G., Pastrana, R., Sherley, J. & Ptashne, M. (1981). Construction of overproducers of the bacteriophage 434 repressor and Cro proteins. *J. Mol. Appl. Genet.* **1**, 139–147.

Levine, A., Bailone, A. & Devoret, R. (1979). Cellular levels of the prophage λ and 434 repressors. *J. Mol. Biol.* **131**, 655–661.

Maniatis, T., Fritsch, E. & Sambrook, J. (1982). *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Maurer, R., Meyer, B. J. & Ptashne, M. (1980). Gene regulation at the right operator (O_R) of bacteriophage lambda. I. O_R1, O_R2 and O_R3: their roles in mediating the effects of repressor and Cro. *J. Mol. Biol.* **139**, 147–161.

Maxam, A. & Gilbert, W. (1977). A new method for sequencing DNA. *Proc. Nat. Acad. Sci., U.S.A.* **74**, 560–564.

Meyer, B. J. (1979). Regulation of λ repressor synthesis directed from P_{RM}. Dissertation, Harvard University, Cambridge, MA.

Meyer, B. J., Maurer, R. & Ptashne, M. (1980). Gene regulation at the right operator (O_R) of bacteriophage lambda. II. O_R1, O_R2 and O_R3: their roles in mediating the effects of repressor and Cro. *J. Mol. Biol.* **139**, 163–194.

Miller, J. H. (1972). *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Mondragon, A. & Harrison, S. C. (1991). The phage 434 Cro/O_R1 complex at 2·5 Å resolution. *J. Mol. Biol.* **219**, 321–334.

Mousset, S. & Thomas, R. (1969). Ter, a function which generates the ends of the mature lambda chromosome. *Nature (London)*, **221**, 242–244.

Ogawa, T., Ogawa, H. Tomizawa, J.-I. (1988*a*). Organization of the early region of bacteriophage ϕ80. *J. Mol. Biol.* **202**, 537 550.

Ogawa, T., Masukata, H. & Tomizawa, J.-I. (1988*b*). Transcriptional regulation of early functions of bacteriophage ϕ80. *J. Mol. Biol.* **202**, 551–563.

Ovchinnikov, Y. A., Guryev, S. O., Kryav, A. S., Monastyrskaya, G., Skryabin, K. G., Sverdlov, E. D., Zakharyev, V. M. & Bayev, A. A. (1979). Primary structure of an *Eco*RI fragment of λimm^{434} DNA containing regions of *cI-Cro* of phage 434 and *cII-O* of phage λ. *Gene*, **6**, 235–249.

Pirrotta, V. (1979). Operators and promoters in the O_R region of phage 434. *Nucl. Acids Res.* **6**, 1495–1508.

Pirrotta, V. & Ptashne, M. (1969). Isolation of the 434 phage repressor. *Nature (London)*, **222**, 541–544.

Poteete, A. R. & Ptashne, M. (1982). Control of transcription by the bacteriophage P22 repressor. *J. Mol. Biol.* **157**, 21–48.

Poteete, A. R., Hehir, K. & Sauer, R. T. (1986). Bacteriophage P22 Cro protein: sequence, purification, and properties. *Biochemistry*, **25**, 251–256.

Ptashne, M. (1971). Repressor and its action. In *The Bacteriophage Lambda* (Hershey, A. D., ed.), pp. 221–237, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Ptashne, M. (1986). *A Genetic Switch*, Cell and Blackwell Scientific Press, Cambridge, MA.

Shean, C. S. & Gottesman, M. E. (1992). Translation of the prophage λ *cI* transcript. *Cell*, **70**, 513–522.

Shih, M.-S. & Gussin, G. (1983). Mutations affecting two different steps in transcription initiation at the phage λ P_{RM} promoter. *Proc. Nat. Acad. Sci., U.S.A.* **80**, 486–490.

Siebenlist, U. & Gilbert, W. (1980). Contacts between *Escherichia coli* RNA polymerase and an early

promoter of phage T7. *Proc. Nat. Acad. Sci., U.S.A.* **77**, 122–126.

Vershon, A. K., Liao, S.-M., McClure, W. R. & Sauer, R. T. (1987). Bacteriophage P22 Mnt repressor. DNA binding and effects on transcription *in vitro*. *J. Mol. Biol.* **195**, 311–322.

Wharton, R. P. (1985). The binding specificity determinants of 434 repressor. Dissertation, Harvard University, Cambridge, MA.

Wharton, R. P., Brown, E. L. & Ptashne, M. (1984). Substituting an alpha helix switches the sequence specific DNA interactions of a repressor. *Cell*, **38**, 361–369.

Wolberger, C., Dong, Y., Ptashne, M. & Harrison, S. C. (1988). Structure of a phage 434 Cro/DNA complex. *Nature (London)*, **335**, 789–795.

Yanish-Perron, C., Viera, J. & Messing, J. (1985). Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC9 vectors. *Gene*, **33**, 103–109.

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