The *bof* Gene of Bacteriophage P1: DNA Sequence and Evidence for Roles in Regulation of Phage *cI* and *ref* Genes†

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The C1 repressor of bacteriophage P1 acts via 14 or more distinct operators. This repressor represses its own synthesis as well as the synthesis of other gene products. Previously, mutation of an auxiliary regulatory gene, *bof*, has been shown to increase expression of some C1-regulated P1 genes (e.g., *ref*) but to decrease expression of others (e.g., *ban*). In this study the *bof* gene was isolated on the basis of its ability to depress stimulation of *Escherichia coli* chromosomal recombination by the P1 *ref* gene, if and only if a source of C1 was present. C1 alone, but not Bof alone, was partially effective. The *bof* DNA sequence encodes an 82-codon reading frame that begins with a TTG codon and includes the sites of the *bof*-1(Am) mutation and a *bof*::Tn5 null mutation. Expression of *ref*::*lacZ* and *cI*::*lacZ* fusion genes was partially repressed in *trans* by a P1 *bof*-1 prophage or by plasmid-encoded C1 alone, which was in agreement with effects on Ref-stimulated recombination and with previous indirect evidence for *cI* autoregulation. Repression of both fusion genes by plasmid-encoded C1 plus Bof or by a P1 *bof*⁺ prophage was more complete. When the C1 source also included a 0.7-kilobase region upstream from C1 which encodes the *coi* gene, repression of both *cI*::*lacZ* and *ref*::*lacZ* by C1 alone or by C1 plus Bof was much less effective, as if *Coi* interfered with C1 repressor function.

Upon infection, temperate bacteriophages respond to physiological signals from their hosts and choose between establishment of stable repressor synthesis (lysogeny) and irreversible commitment to lytic-gene expression. Bacteriophage P1 maintains stable lysogeny in host bacteria, as other temperate phages do, by binding its primary repressor, C1, to promoter-operators for lytic genes (41). Prophage P1 is a (unit-copy) plasmid rather than a part of the host chromosome, and its regulatory apparatus is unusually complicated; the C1 repressor acts at numerous sites scattered throughout the genome (including the *cI* gene itself). P1 differs from most temperate phages in two major respects.

In contrast to phage λ and its relatives, whose two promoter-operators each contain three symmetric sequences that bind repressor dimers, there are at least 14 C1-binding sites in 10 different regions of the P1 genome (5). The C1 operators, all of which include an asymmetric sequence that is identical or similar to the consensus sequences ATTGC TCTAATAAATTT, are numbered according to their location on the P1 map (40) (Fig. 1) (e.g., Op2, Op21). Operators near the same map coordinate are designated with letter suffixes (e.g., Op99a, Op99e). The P1 operators are almost all oriented in the same direction relative to associated promoters, but their locations relative to the putative -35 and -10 sequences vary considerably (2, 5, 7, 11, 37). (The C1 repressor of the closely related but heteroimmune phage P7 appears to be identical to the P1 repressor [4, 34].)

The λ repressor controls its own maintenance synthesis during lysogeny by means of the *o_R* operators, which overlap the maintenance promoter region (23). However, a critical factor in the lysis-lysogeny decision is initiation of transcription of the λ repressor gene at an establishment promoter, which is not associated with any repressor-binding site and is positively regulated by the λ cII protein (38). In contrast, both of the promoters which might logically be expected to

initiate P1 *cI* transcription are associated with C1-binding sites (7). Thus, P1 must employ different regulatory mechanisms than λ during establishment of lysogeny and most likely during maintenance as well.

The actions of C1 repressor at its own and other genes are modulated by auxiliary elements, which are organized into three regions (41). The *immC* region, at coordinates 99 to 0, includes the *cI* gene and the following upstream elements: two promoter-operators, another operator, and three small open reading frames. The order (counterclockwise on the P1 map) is P-Op99d *orf*-2 *orf*-3 *orf*-4 Op99c P-Op99ba *cI*. The *virC* mutation, which maps upstream from these elements and appears to make expression of at least one of the *orf* genes constitutive (10), might be considered part of *immC*. Overexpressed *orf* protein is thought to cause the phenotype of P1 *virC* by acting at the level of C1 function (41) rather than at the level of *cI* gene expression (10, 25).

The *immI* region (coordinates 50 to 52) is responsible for the synthesis and control of a function or functions (*ant* [34], *reBA,B* [25]) that antagonize C1 and for P1-P7 heteroimmunity (27). Expression of *ant* and *reb* is regulated by the *c4* gene. Although the mechanism of antagonism is not well understood, it appears not to involve direct inactivation of C1 by Ant and Reb, unlike the antirepressor mechanism of phage P22 (32).

The *immT* region is defined by the *bof* gene (33) at coordinate 9.5, and its product is yet another modulator of C1 action. The Bof⁻ phenotype is complicated and somewhat paradoxical, suggesting a correspondingly complex mechanism of action. A positive role for Bof in expression of the C1-controlled P1 *ban* gene, which encodes an analog of the *Escherichia coli* DnaB protein, was suggested by the inability of second-site mutants of certain prophages (P1 *bac*-1), normally Ban constitutive, to express Ban activity. The Ban⁻ phenotype of the doubly mutant P1 *bof*-1 *bac*-1 prophages suggested the designation "ban on function" (*bof*) (33). (*bac*-1 is a mutation in C1-binding site Op72a that is associated with the *ban* promoter [11].) In contrast, a negative role for Bof in the regulation of some P1 genes was

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FIG. 1. Regulatory circuits of bacteriophage P1. The positions of important regulatory elements in the *immC* (*c1*, *coi*), *immI* [*ant*(*rebA*, *B*), *c4*] and *immT* [*bof*(*lxc*, *c6*)] regions and some regulatory targets are indicated. The positions relative to P1 map coordinates 0 to 100 (inside the circle) are from reference 40; the location of the putative P1 *ssb* gene (14) is not known. The heavy solid arrows indicate C1-mediated negative-control interactions inferred from the presence of C1 operators and in some cases (*ban* [17], *ref* [36], *c1* [this work]) directly demonstrated. The light solid arrows indicate negative-control or antagonistic interactions demonstrated by or inferred from existing data. The dashed arrow indicates a possible positive-control interaction, based on the Ban-constitutive phenotype of *bof-1* prophages (33).

suggested by the markedly increased levels of the following two activities in P1 *bof-1* prophages: Ref, which stimulates certain RecA-dependent homologous recombination processes in *E. coli* chromosomes (16, 36) and plasmids (15a), and P1 Ssb, which suppresses the defects of *E. coli ssbA*(Ts) mutants at nonpermissive temperatures (14, 36). (P1 *lxc* mutations confer the same phenotypes and appear to be allelic with *bof-1* [14].) The Bof effects on immunity establishment and maintenance appear to be similarly contradictory. The immunity of P1 prophages against superinfection (by P1 *vir*^s and P7) seems to be weakened by Bof (31, 33), but the stability of P1 *c1.100*(Ts) prophages appears strengthened by Bof (33). *c6* may be allelic with *bof* (26).

Although C1 is necessary for regulation of *ref* gene expression, the Ref-constitutive and Ssb-constitutive phenotypes of P1 *bof-1* and *lxc* prophages demonstrate that C1 is not sufficient (36). In this study we used depression of Ref recombination-stimulation activity in the presence of C1 as an assay for isolation of the *bof* gene and determined the *bof* DNA sequence. Using the isolated *bof* gene and *ref::lacZ* and *c1::lacZ* fusion genes, we found that Bof is a negative effector of both *c1* transcription and *ref* transcription, if and only if C1 is also present.

MATERIALS AND METHODS

Bacteria, bacteriophages, and plasmids. The bacteria, bacteriophages, and plasmids which we used are described in Tables 1 through 3. Stocks of λ bacteriophages were prepared by growth to confluent lysis on S plates (see below), as described previously (30). Stocks of P1 bacteriophages were prepared by growth to confluent lysis on R plates (see below) (20). Lysogens of P1 CmO phages were prepared by spotting

TABLE 1. Bacterial strains

Strain	Genotype	Source or reference
C600	<i>lacY1 leuB6 supE44 thr-1 thi-1 tonA21</i>	1
JM101	$\Delta(lac-proAB)$ <i>thi supE(F' tra36 proAB lacI⁺ lacZ ΔM15)</i>	39
KS391	HfrH <i>lacZ</i> M5286 (ϕ 80dIII <i>lacZ</i> BK1)	15
MPh30	$\Delta(argF-lacZ)$ U169 <i>phoA::Tn5</i>	R. Wolf
TSS301	MPh30(λ TSS301)	This work
TSS321	MPh30(λ TSS321)	This work

phage lysates onto appropriate bacterial lawns and streaking turbid centers onto LB plates (see below) supplemented with chloramphenicol (10 μ g/ml). λ bacteriophages encoding *ref::lacZ* and *c1::lacZ* gene fusions were prepared by plate stock growth of λ RS88 on strain MPh30(P1 *c1*⁺ *r*⁻ *m*⁻) cells harboring appropriate plasmids and were identified as prophages in strain MPh30 cells spread onto X-Gal plates (see below) (blue colonies). Plasmids were extracted from bacteria by rapid-boiling (13) or alkaline-lysis techniques (19) and were purified by equilibrium sedimentation (4.5 h at 90,000 rpm [340,000 $\times$ g]) in CsCl-ethidium bromide, using 3.5-ml tubes and a model TL-100 table top ultracentrifuge (Beckman Instruments, Inc.).

Media and buffers. TBE buffer contained 89 mM Tris base, 89 mM boric acid, and 2 mM disodium EDTA (pH 8.0). TE buffer contained 10 mM Tris hydrochloride and 1 mM disodium EDTA (pH 8.0). Z buffer contained 60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 10 mM KCl, 1 mM MgSO₄, and 50 mM 2-mercaptoethanol (pH 7.0). TBY broth contained 1% tryptone (Difco Laboratories), 0.5% yeast extract (Difco), and 1% NaCl. M9 medium contained 42 mM Na₂HPO₄, 22 mM KH₂PO₄, 8.5 mM NaCl, 18.7 mM NH₄Cl, 10 mM CaCl₂, 0.2% glucose, and 0.001% thiamine. LB plates contained TBY broth solidified with 1.5% agar. MacConkey-lactose plates contained 4% MacConkey agar base (Difco) and 1% lactose. R plates contained 1% tryptone (Difco), 0.1% yeast extract, 0.8% NaCl, 2 mM CaCl₂, 0.1% glucose, and 1.2% agar. S plates contained 1% tryptone (Difco), 0.5% NaCl, 0.075 mM CaCl₂, 0.004 mM FeCl₃, 10 mM MgSO₄, 0.3% glucose, 0.001% thiamine, and 1% agar. TCMB plates contained 1% Trypticase (BBL Microbiology Systems), 0.5% NaCl, 10 mM MgSO₄, 0.0001% thiamine, and 1.1% agar. X-Gal plates were LB plates that were supplemented with 40 μ g of 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside per ml. Antibiotics were used in plates at the following concentrations: ampicillin, 75 μ g/ml; neomycin, 50 μ g/ml; and chloramphenicol, 10 μ g/ml.

General recombinant DNA techniques. Restriction endonucleases were purchased from New England BioLabs, Inc., United States Biochemical Corp., or Bethesda Research Laboratories, Inc. T4 DNA ligase was obtained from New England BioLabs, and exonuclease III, S1 nuclease, and DNA polymerase I (large [Klenow] fragment) were purchased from Bethesda Research Laboratories. All enzymes were used as specified by the manufacturers. Nested deletions were generated by exonuclease III digestion at 5'-overhang restriction enzyme termini (in the presence of exonuclease III-resistant 3'-overhang termini), as described by Henikoff (12). Electrophoresis of DNA fragments was performed in 0.7 to 1.0% agarose or 12% polyacrylamide gels in TBE buffer; fragments were isolated from gels by electroelution techniques. Bacteria were transformed with plasmids by using the CaCl₂ technique (18) or by using the following adaption of other procedures (19): cells were suspended in

TABLE 2. Bacteriophage strains

Strain	Genotype and/or remarks	Source or reference
λ RS88	' <i>bla</i> ' <i>lacZ lacY lacA imm</i> ⁴³⁴ <i>ind</i> ⁻	29 ^a
λ 301	' <i>bla</i> ' <i>Tl</i> ₄ P1: <i>ref::lacZ lacY lacA imm</i> ⁴³⁴ <i>ind</i> ⁻ (λ RS88 $\times$ pTS301 recombinant) ^b	This work ^a
λ 321	' <i>bla</i> ' <i>Tl</i> ₄ P1: <i>cl::lacZ lacY lacA imm</i> ⁴³⁴ <i>ind</i> ⁻ (λ RS88 $\times$ pTS321 recombinant)	This work ^a
P1 Cmo <i>r</i> ⁻ <i>m</i> ⁻	Lacks P1 restriction modification	M. Gottesman
P1 Cmo <i>cl.100 ref::Tn5-101</i>	Ref ⁻	36
P1 Cmo <i>bof-1</i>	Derepressed for <i>ref</i> , putative <i>ssb</i>	N. Sternberg ^c
P1 Cmo <i>lxc-11</i> ^d	Derepressed for <i>ref</i> , putative <i>ssb</i>	L. Rosner

^a See Fig. 4.^b *Tl*₄ is a tandem tetramer of the *E. coli rrnB* transcription terminator that is oriented so as to block transcription of *lacZYA* from upstream promoters.^c See reference 33.^d Phage P1 Cmo *lxc-11* appears to have undergone a rearrangement that inactivated the *bof* gene. *Bam*HI restriction fragments 3 and 4 (P1 coordinates 3 to 12 and 18.5 to 31.5, respectively) and *Sma*I fragments 2 and 3 (P1 coordinates 10 to 29 and 97 to 100, respectively) are altered, and there is a novel joint that fuses *bof* DNA that is 3' with respect to bp 656 to P1 DNA of unknown origin.

an equal volume of a solution containing cold 0.1 M MOPS (morpholinepropanesulfonic acid) (pH 6.5), 50 mM CaCl₂, 10 mM RbCl₂, and 15% glycerol, incubated on ice for 1 h; centrifuged, and then suspended in 0.1 volume of the same buffer. The cells were frozen at -80°C or were used immediately.

DNA sequence determinations. The dideoxy chain termination method of Sanger et al. (24) was used with duplex plasmid DNA primed with synthetic oligonucleotide primers, as described by Zhang et al. (42), using modified phage T7 DNA polymerase (Sequenase; United States Biochemical Corp.) and [³⁵S]dATP (New England Nuclear Corp.). Sequencing reaction mixtures were electrophoresed in 6 to 8% polyacrylamide gels. Gels were rinsed in 5% methanol-5% acetic acid, transferred to Whatman 3MM filter paper, and dried. X-ray film was exposed to the filter paper for 20 to 70 h, as needed.

The sequence of the entire 0.93-kilobase (kb) *Bam*HI-*Dra*I fragment of plasmid pTS882 was obtained by using a variety of primers; plasmid templates were derived by subcloning various fragments. Below, the approximate regions sequenced and the direction of dideoxy synthesis are indicated in parentheses; e.g., the (0.44-0.72) sequence is the region from position 0.44 to position 0.72 synthesized from left to right (Fig. 2), yielding 280 base pairs (bp) of sequence. The pUC universal primer CAGCACTGACCCTTTTG, corresponding to 17 nucleotides of the *lacZ* sequence downstream from the multilinker region of pUC plasmids, was used with a nested series of deletion derivatives that spanned the entire fragment (generated from pTS881 by using the Henikoff [12] technique [*Bam*HI-*Sal*I restriction, exonuclease III digestion of a 3'-ended strand at the *Bam*HI end, treatment with S1 nuclease and DNA polymerase I [Klenow fragment],

TABLE 3. Plasmids

Plasmid	Description and/or remarks ^a	Source or reference
pACYC184	Tc ^r Cm ^r	3
pAClac19	<i>Av</i> II- <i>Bgl</i> II fragment from M13m19 (encoding <i>lacPO</i> , polylinker, and additional 5' segment of <i>lacZ</i> replacement inserted into <i>Hind</i> II- <i>Bam</i> HI sites [<i>tet</i> gene] of pACYC184)	T. Schaefer ^b
pBR325	Ap ^r Cm ^r	22
<i>pcI</i> Δ r	Encodes Op99ba <i>cl</i> of phage P1	N. Sternberg ^b
pMV1w	<i>Pvu</i> II- <i>Bcl</i> II fragment of P1 <i>c</i> ⁺ in pJF118EH, such that <i>p</i> _{lac} drives (in order) <i>orf</i> -2, <i>orf</i> -3, <i>orf</i> -4, and <i>cl</i> ^c	H. Schuster ^b
pPR110	Encodes (λ cl857 <i>p</i> _l)-multilinker- <i>lacZ</i>	35
pRS415	Encodes <i>bla</i> <i>Tl</i> ₄ (no promoter) <i>lac</i> 'ZYA' ^d	29
pTS-4-6	P1 <i>Bam</i> HI fragment 4 in <i>Bam</i> HI site of pPR110, Bof ⁺	This work
pTS301	<i>Eco</i> RI- <i>Sau</i> 3A fragment of pUC19r81, encoding <i>ref</i> promoter-operator, attenuator, and 21 N-terminal amino acids, in <i>Sma</i> I site of pRS415 ^f	This work ^e
pTS321	<i>Ball</i> - <i>Ball</i> fragment of pMV1w, encoding Op99ab <i>p</i> _{cl} , in <i>Sma</i> I site of pRS415	This work ^e
pTS871	P1 <i>Bam</i> HI fragment 4 in <i>Bam</i> HI site of pACYC184, Bof ⁺ Tc ^s	This work ^g
pTS872	<i>Bgl</i> II- <i>Bam</i> HI fragment of pTS871 in <i>Bam</i> HI site of pACYC, Bof ⁺ Tc ^s	This work ^g
pTS873	8-bp <i>Bam</i> HI linker [d(CGGATCCG)] in unique <i>Sma</i> I site of pTS872, Bof ⁺ Tc ^{sh}	This work ^g
pTS874	Bof ⁺ derivative of pTS873	This work ^g
pTS8745	<i>bof::Tn5</i> derivative of pTS874, Bof ⁻ Tc ^s	This work ^g
pTS881	Bof ⁺ derivative of pTS874, pAClac19	This work ^g
pTS882	Bof ⁺ derivative of pTS881, pAClac19	This work ^g
pTS891	Bof ⁺ derivative of pTS882, pUC19	This work ^g
pTS894	Bof ⁺ derivative of pTS882, pUC19	This work ^g

^a Tc^s, Tetracycline sensitive; Tc^r, tetracycline resistant; Ap^r, ampicillin resistant; Cm^r, chloramphenicol resistant.^b Unpublished data.^c See reference 8.^d See Table 2, footnote b.^e See Fig. 5.^f See reference 37.^g See Fig. 2.^h d(CGGATCCG) was obtained from New England BioLabs.

FIG. 2. Localization of the *bof* gene. In each case, constructions containing P1 DNA fragments were tested for Bof activity (the ability, in concert with the *cl*-encoding plasmid *pcIΔr*, to suppress Ref-stimulated *lac*⁻ × *lac*⁻ chromosomal recombination in strain BW2060 [assayed as frequency of Lac⁺ papillae per colony] [Table 4]) and analyzed by restriction and electrophoresis. All plasmids shown except pTS8745 expressed full Bof activity. Left to right corresponds to the counterclockwise direction on the P1 map, from the *Bam*HI site at coordinate 12 to the *Bam*HI site at coordinate 2.5 (Fig. 1). Plasmids were constructed as follows: pTS871, isolation of 9.5-kb P1 *Bam*HI fragment 4 and insertion of this fragment into the unique *Bam*HI site in the *tet* gene of pACYC184; pTS872, isolation of P1 *Bam*HI fragment 4, *Bgl*II restriction, and insertion of the 4.5-kb *Bam*HI-*Bgl*II fragment into the *Bam*HI site of pACYC; pTS873, ligation of an 8-nucleotide *Bam*HI linker(s) into the unique *Sma*I site of pTS872 (XB); pTS874, *Bam*HI restriction of pTS873, purification of the larger fragment, and religation; pTS8745, infection of C600(pTS874) with λ::Tn5 *b221 Oam Pam*, selection for stable Cm^r Nm^r colonies, extraction of plasmids, transformation of strain C600, and selection for chloramphenicol and neomycin resistance (approximate localization of the Tn5 insertion point in the 7.9-kb fragment by *Bam*HI and *Hind*III restriction mapping [one and two cuts in Tn5, respectively]); pTS881, *Bam*HI-*Eco*RV (site in pACYC184 188 bp from the *Bgl*II-*Bam*HI joint) restriction of pTS874, isolation of smaller (2.5-kb) DNA fragment, and replacement cloning into *Bam*HI and *Hinc*II sites of pAC1ac19; pTS882, isolation of smaller *Hind*III-*Bam*HI fragment from pTS874; *Dra*I restriction and isolation of (926-bp) *Dra*I-*Bam*HI subfragment, and insertion into *Hinc*II and *Bam*HI sites of pAC1ac19. Abbreviations of restriction-site designations: B, *Bam*HI; BG, *Bgl*II; SM, *Sma*I; RV, *Eco*RV; DR, *Dra*I; X, destruction of existing recognition site. The heavy lines indicate P1 DNA, the light lines indicate pACYC184 DNA, and the dotted lines indicate pAC1ac19 DNA. Sizes are the sizes of the Bof-encoding fragments indicated by the heavy lines and correspond to DNA sequence information in the case pTS882 and to estimates based on electrophoretic mobility in other cases.

ligation) to generate the (0.0-0.9) sequence. The pUC universal primer and the reverse primer AACAGCTATGAC CATG (corresponding to 16 nucleotides of sequence that is just upstream from the multilinker region in pUC plasmids and spans the *lacZ* translation start signal) were used with the *Bam*HI-*Fsp*I fragment of pTS882 replacement inserted into the *Bam*HI and *Hind*II sites of pUC19 to generate the (0.70-0.45) and (0.0 to 0.26) sequences, respectively, and with the *Pst*I-*Fsp*I fragment (from plasmid pTS882) inserted into the *Pst*I and *Hinc*II sites of pUC19 to generate the (0.66-0.93) and (0.93-0.67) sequences, respectively. The pUC universal primer was used with plasmid pTS882 to generate the (0.0-0.25) sequence. The 17-nucleotide primer (GGGAAAGGTTCCGTTTC), corresponding to a conserved sequence in the right and left IS50 arms of Tn5, was used with fragments containing, respectively, the *bof* DNA to the left of the insertion (associated with the left *Sal*I one-half of Tn5) and the *bof* DNA to the right of the insertion (associated with the right [neomycin resistance-encoding] *Sal*I one-half of Tn5), after the fragments were generated by deleting the *Sal*I-*Sal*I fragment encoding the right (neomycin resistance) one-half and adjacent P1 and pACYC184 DNA from pTS8745 and then inserting that fragment into pACYC184, to obtain the (0.56-0.90) and (0.56-0.33) sequences, respectively. Primers TS4 (GCCAGTCCCTGA AGA) and TS3 (TGATGATCTGCTCCC), corresponding to locations in the *bof* gene region, were used with plasmid pTS882 to generate the (0.43-0.72) and (0.73-0.41) sequences, respectively.

Measurement of β-galactosidase expression by fusion genes.

(i) **Uninduced plasmids and prophages.** M9 medium supplemented with 5% TBY broth, 0.02% Casamino Acids, 0.2%

glucose, and appropriate antibiotics for selecting for plasmid maintenance was inoculated directly with colonies of bacteria (strain MPh30 derivatives), and cultures were grown for about 8 h with shaking at 32°C. At an A₆₀₀ of about 0.5, the cultures were diluted in Z buffer (usually 1:2 or 1:10), and 1-ml portions were mixed with 0.05-ml portions of 0.1% sodium dodecyl sulfate plus 0.1-ml portions of CHCl₃ in 1.9-ml microcentrifuge tubes. At time zero, samples were mixed with 0.2 ml of 0.4% *ortho*-nitrophenyl-β-D-galactopyranoside and warmed to 37°C. After appropriate incubation times (usually 5 to 20 min), samples were mixed with 0.5-ml of 1 M NaCO₃ and centrifuged for 1 min in a Microfuge (Beckman Instruments). Supernatants (about 1 ml) were transferred to disposable microcuvettes for determinations of A₄₂₀ (*ortho*-nitrophenyl-β-D-galactopyranoside absorbance) and A₅₅₀ (cell turbidity correction). β-Galactosidase units were calculated as described by Miller (20).

(ii) **IPTG-induced pMV1w.** M9 medium containing the supplements described above plus isopropylthio-β-D-galactopyranoside (IPTG) was inoculated with portions of colonies suspended in broth or sterile saline, and cultures were grown up and assayed as described above. Multiple samples of the same suspension were used for replicate determinations.

Measurement of *lac*⁻ × *lac*⁻ recombination. (i) **Lac⁺ papillation assay.** For the Lac⁺ papillation assay bacteria (strain KS391 derivatives) were streaked onto MacConkey-lactose plates and grown for 48 h at 32°C; then the Lac⁺ papillae in about five to seven colonies were counted.

(ii) **Assay for preexisting Lac⁺ colonies.** In the assay for preexisting Lac⁺ colonies bacteria (strain KS391 derivatives) were grown in TBY broth for about 18 h, and the cultures were serially diluted in 0.85% NaCl and spread onto

TABLE 4. Suppression of Ref activity by *trans*-acting P1 genes

C1 source	Bof source	Relative frequency of Lac ⁺ cells in Ref ⁺ bacteria ^a
None ^b	None ^b	25
<i>pcIΔr</i>	None ^b	15
None ^b	pTS871 ^b	21
<i>pcIΔr</i>	pTS871	0.9
P1 c ⁺	P1 c ⁺	0.6

^a The frequency of *lac*⁻ × *lac*⁻ chromosomal recombination in plasmid-bearing derivatives of strain BW2060, which is KS391(λ-P1 *Bam*HI fragment 8), was assayed by measuring the frequency of preexisting Lac⁺ cells in overnight cultures, as described in Materials and Methods. A relative frequency of 1.0 corresponds to the value for strain KS391 (7 × 10⁻⁴).

^b Vector plasmid(s) only.

LB plates (to determine cell concentrations) and onto MacConkey-lactose plates (about 10⁵ to 10⁶ colonies per plate) (to determine Lac⁺ cell concentration); the plates were incubated for 20 h at 32°C.

RESULTS

Requirement for both *cI*-encoding and *bof*-encoding DNA fragments to suppress Ref recombination stimulation activity. Previous observations that P1 *cI*⁺ *bof*-1 mutants were derepressed for Ref activity (36) and that *cI*-encoding DNA segments did not repress *ref* expression in *trans* (S. D. Lu, D. Lu, and M. E. Gottesman, unpublished data; B. E. Windle, Ph.D. thesis, University of Maryland-Baltimore County, Catonsville, 1986) together suggested that in addition to C1, the product of the *bof* gene or the product of one or more genes controlled by the *bof* gene was required to fully repress *ref* gene expression. The *bof*-1 mutation maps to P1 coordinate 9.5 (33, 41) (Fig. 1). We tested wild-type P1 *Bam*HI fragment 4, which spans coordinates 3 through 12, for its ability to complement a P1 *bof*-1 prophage for repression of *ref* expression when the fragment is inserted into plasmid vector pPR110 (35). Ref activity was estimated indirectly as stimulation of *lac*⁻ × *lac*⁻ chromosomal recombination (manifested as increased frequency of Lac⁺ papillae on colonies of appropriate derivatives of strain KS391 [36]). Under conditions (see Materials and Methods) such that strain KS391 averaged about one Lac⁺ papilla per colony, strains KS391(P1 *bof*-1) and KS391(P1 *lxc*-11) averaged over 30 Lac⁺ papillae per colony. In the presence of P1 *Bam*HI fragment 4 (on a multicopy plasmid), the latter two lysogens showed only one to two and two to four papillae, respectively; i.e., P1 *Bam*HI fragment 4 sufficed for complementation.

To further define requirements for regulation of *ref* expression, we inserted the 9.5-kb P1 *Bam*HI fragment 4 into pACYC184 (plasmid pTS871) and tested the effect of this plasmid and a compatible plasmid, *pcIΔr* (N. Sternberg, unpublished data), which bears only the P1 *cI* gene and the immediately associated promoter-operator (P-Op99ba [7]). Ref activity was assayed as the frequency of Lac⁺ cells in cultures of appropriate strain KS391 derivatives. Although C1 function alone partially depressed Ref activity, C1 and a source of Bof were necessary (and sufficient) for full repression (Table 4). This assay was not precise enough for the small decrease in Ref activity in the presence of plasmid-borne P1 *Bam*HI fragment 4 without C1 to be considered significant.

Localization of the *bof* gene. The localization of the *bof* gene by Tn5 mutagenesis and deletion experiments is summarized in Fig. 2. Bof activity (defined as suppression, in the presence of *lac*⁻ × *lac*⁻ chromosomal recombination stim-

ulated by a λ-P1:B8 prophage [P1 *Bam*HI fragment 8 inserted into phage λD69 {36}]) was expressed by a series of subfragments of P1 *Bam*HI fragment 4 inserted into plasmids (see Fig. 2 legend). All of the constructions shown in Fig. 2 except pTS8745 expressed full Bof activity. We constructed a series of unidirectional deletions of pTS5881 DNA, beginning at the point where *Bam*HI linkers had been inserted at the *Sal*I site (designated XB; see structure of pTS5872 in Fig. 2) and extending increasingly farther into the P1 sequence. Bof activity was eliminated when these deletions extended about 0.7 kb or more from the XB site (estimated by restriction analyses). In pTS8745, the Tn5 insertion, which eliminated Bof activity, was determined by restriction analyses to be 0.6 to 0.9 kb from the XB site.

DNA sequence of the *bof* gene. Direct dideoxy plasmid DNA sequencing, in which we used a variety of oligonucleotide primers, was used to identify the *bof* gene, as described above. There were five independent sources of *bof*⁺ sequence information for the critical region that encodes the presumptive translation start codon (see below). The sequence of the *bof*-1(Am) gene in plasmid pMV15RD (kindly provided by H. Schuster) was determined by extension of primer TS3.

The DNA sequence of the *bof* gene region of phage P1 is shown in Fig. 3; this sequence is numbered counterclockwise from the center of the *Sma*I site near P1 map position 10. (The *Bam*HI linker nucleotides in plasmid pTS873 and derivatives are not numbered.) The DNA sequence between bp 388 and 931 was determined by Schuster and colleagues (M. Heirich, M. Velleman, and H. Schuster, unpublished data) and is in complete agreement with the sequence which we determined. We identified the open reading frame beginning with the TTG at bp 541 to 543 as *bof* for the following reasons: (i) the TAC at bp 553 to 555 in *bof*⁺ is a TAG in *bof*-1(Am); (ii) there is a TGA at bp 511 to 513 in frame with the TAC at bp 553 to 555 (there is no ATG between bp 513 to 553, but the TTG at bp 541 to 543 is preceded by a reasonable ribosome-binding site [28]); and (iii) the reading frame includes bp 601 to 609, which flank (in duplicate) the *bof*::Tn5 insertion. The *bof* reading frame terminates with a TAA at bp 787 to 789 and thus encodes an 82-amino-acid polypeptide, in agreement with the 9,600-M_r protein identified as Bof by Schuster and co-workers (unpublished data). About 1% of *E. coli* proteins initiate translation at a TTG codon (9). Schuster and co-workers (M. Velleman, M. Heirich, A. Günther, and H. Schuster, unpublished data) have identified the TATGAG at bp 482 to 487 as a transcription signal (-10) required for *bof* expression.

Bof-plus-C1-mediated repression of *ref*::*lacZ* expression. Inhibition of Ref stimulation of *E. coli lac*⁻ × *lac*⁻ recombination by Bof activity in the presence of C1 activity could in principle reflect interference with the recombination process rather than repression of *ref* gene transcription. Furthermore, the relationship between *ref* gene transcription and the frequency of Ref-stimulated recombination is not known. In order to measure directly the effect of Bof and C1 on *ref* transcription, we constructed a *ref*::*lacZ* fusion gene (Fig. 4). The 0.25-kb *Bam*HI-*Sau*3A fragment from pUC19r81 (37) encodes a few vector base pairs plus the following segments: the *ref* promoter-operator region (37) dual tandem promoters (C1 recognition sequence); the *ref* attenuator (37); and the first 65 bp of the *ref* reading frame. This fragment was fused (out of frame) to the *Bam*HI site of pRS415 (29), thus placing the *ref* transcription initiation signals upstream from a promoterless *lacZYA* segment (a few base pairs of the *trp* sequence [lacking expression

GGGTAACG TGGATTATCG AGACAAAACA CAACGTGGCG GACGTTCTGG CCGTCATCCA ACAATACGCA TAACAGGAGC GCCCGGTTTC GGTCGGCAT 100

AATATGGCCA CACTATCTGA TACAATAAAA CCGAATAAAA CATATCTTGA GCGGCTACTG CGTACGGCAT TATTAGGAAA GACAGAAGAC GAATACGTTG 200

ATTCTTCTCT GTCAGGGCTA CGCGGGCGAT TACTGAAAAA TCCCGGCTG TACCGCAGCT ATGGCCGATA CTGGCGGAAA TTAATAAATT ATTACTGGAG 300

CGACGGTTAT GGTAATTTTC GTGCTCTCGT TGACCGTGAC GTTCGCAGAT TTTACCGTTA TGACCGCCCG GCGCTAACAC TCATAGCCGC GACGCTCTAC 400

AGCCATGAGC GTTTTGATAA TGGTCAGATA TACTCAGCCT GGCATTACT GCCAGTCCCT GAAGAAGTTG ACGACCAGGA CTATGAGTTT GAGTCTTACG 500

ATTGGAAGT TGAAGCCTTG GCACAGGCTG GAGAGAAAA TTGAAAAAGC GATACTACAC AGTAAAGCAT GGGACGCTAC GAGCATTACA AGAGTTTGCT 600

..... TER ?LysLysA rgTyrTyrTh rValLysHis GlyThrLeuA rgAlaLeuGl nGluPheAla

GACAAGCATA ACGTTGAGGT CGCGAGGGAA GGGGGAAGTA AAGCTCTGCG CATGTACCGT CCGGACGGGA AATGGCGGAC GGTCTGCGAT TTCAAACAA 700

AspLysHisA snValGluVa lArgArgGlu GlyGlySerL ysAlaLeuAr gMetTyrArg ProAspGlyL ysTrpArgTh rValValAsp PheLysThrA

ACAGTGTTCG CCAGGGCGCTG CGTACCGGGG CATTGGAAGA ATGGGAGCAG ATCATCATAG ATAATGCATT GCTTCTCAAT GCGGATTAAA CTTCGCCAAA 800

snSerValPr oGlnGlyVal ArgAspArgA laPheGluGl uTrpGluGln IleIleIleA spAsnAlaLe uLeuLeuAsn AlaAspTER

TTAGGGCTGT TTGCTCACCG AGCATCGCTC AAAGAAGCAC GATTCTTCAA ACATATAGAT AGTGATAGTG CCACAACCTC TGGCTCTAAC GGGCTGGGGA 900

GGCGGGGCTT TGTGTGTAAT TGATCTGTT T 931

FIG. 3. Sequence of the *bof* region of bacteriophage P1. The DNA sequence of 931 bp of P1 DNA, beginning at the *Sma*I site near P1 map coordinate 10, was determined as described in Materials and Methods. We did not directly demonstrate translation of the presumptive TTG initiation codon or the activity of the putative translation (28) signal (ribosome-binding [RBS]). The C-to-G mutation in *bof-1*(Am) and the repeated sequence (dots) that flanks the insertion in *bof::Tn5* are indicated. This sequence has been placed in the GenBank-European Molecular Biology Laboratory database under accession number M33224.

signals] are 5' with respect to *lacZYA*). The fusion gene was transferred to phage λ RS88 (29) by homologous recombination, and the λ *ref::lacZ* phage was used to lysogenize Δ *lac* strain MPh30.

MPh30(λ *ref::lacZ*) lysogens synthesized only about one-sixth as much β -galactosidase as *lacZ*⁺ bacteria (data not shown). (There is a rho-independent attenuator in the *ref* leader sequence [37].) Expression of *ref::lacZ* was repressed twice as well by Plc⁺ prophage as by P1 *bof-1* and P1 *lxc* prophages (Table 5), which is in qualitative agreement with the effects of these prophages on Ref-stimulated recombination (36). P1 *cl.100* prophages suppressed *ref::lacZ* expression much less effectively than Plc⁺ prophages, which is also in agreement with the results of recombination studies; the P1 *cl.100* prophage data were highly variable (sometimes cultures showed partial lysis), but supported the notion that *ref* is partially derepressed in P1 *cl.100* prophages (36).

We tested a plasmid encoding an isolated *bof* gene and two plasmid sources of the *cl* gene for regulation of *ref::lacZ* expression (Table 6). C1 alone (in the presence of *bof::Tn5* plasmids) resulted in partial repression (Table 6); the addition of Bof increased repression. The combination of Bof plus C1 was about as effective as an intact Plc⁺ prophage (Tables 5 and 6).

Modulation of *cl* expression and C1 activity. C1 appears to regulate its own synthesis (21, 41). The increased superinfection immunity shown by *bof-1* mutants (33) suggested that Bof might be a coregulator of *cl* expression. We constructed a *cl::lacZ* fusion gene (Fig. 4) and assayed for Bof and C1 effects. The *cl* portion was a 74-bp *Bal*I fragment that encoded associated near-canonical (Op99a) and degenerate (Op99b) C1 operators (Fig. 4) and no upstream P1 DNA. A Plc⁺ prophage suppressed *cl::lacZ* expression about four times as well as a P1 *bof-1* prophage (Table 7), indicating that

FIG. 4. Construction of *ref::lacZ* and *cl::lacZ* fusion phages. The following steps were used for *ref::lacZ* construction: *Eco*RI digestion of plasmid pUC19r81 (*Bam*HI-*Bam*HI fragment from *ref*-encoding plasmid pPR110r81 [37], inserted into the pUC19 *Bam*HI site) and electrophoretic isolation of the smaller (*ref*-encoding) (0.7-kb) fragment; digestion of the 0.7-kb fragment with *Sau*3A and electrophoretic isolation of two subfragments, one of which was expected to encode the *ref* promoter-operator region; replacement insertion of each subfragment into pRS415 (29) at the *Bam*HI site of the latter and screening of transformants of MPh30(P1 c⁺ r⁻ m⁻) for the Lac⁺ phenotype to identify *ref::lacZ* constructions (e.g., pTS301) in which *lacZ* was expressed via *ref* transcription signals; verification of novel joints in plasmid pTS301 by dideoxy sequencing, using a *lacZ* sequence primer with a sequence complementary to pUC19 universal primer; preparation of λ RS88 plate growth stock on MPh30(P1 c⁺ r⁻ m⁻)(pTS301) cells to accomplish *bla* $\times$ *'bla* and *lacZYA* $\times$ *lacZYA* recombination between λ RS88 and pTS301; and lysogenization of MPh30 cells with the phage plate stock and spreading onto X-Gal plates for identification of MPh30(λ TS301) lysogens (light blue colonies). The following steps were used for *cl::lacZ* construction: *Bal*I restriction of plasmid pMV1w (*Pvu*II-*Bcl*I subfragment from the *immC* region of P1 c⁺, inserted into expression vector pJF118EH [6; H. Schuster, personal communication]) and isolation of a 74-bp fragment encoding P-Op99ba; insertion into the *Sma*I site of pRS415 and identification of Lac⁺ construction pTS321 by transformation of MPh30(P1 c⁺ r⁻ m⁻) cells; verification of the structure of the P-Op99ba-*lacZ* novel joint by direct dideoxy sequencing on plasmid pTS321, using universal synthetic primer; preparation of λ RS88 plate growth stock on MPh30(P1 c⁺ r⁻ m⁻)(pTS321) cells to accomplish *'bla* $\times$ *bla* and *lacZYA* $\times$ *lacZYA* recombination; and infection of MPh30 cells with phage plate stock and identification of Lac⁺ MPh30(λ TS321) lysogens as blue colonies on X-Gal plates.

TABLE 5. Regulation of *ref::lacZ* expression by P1 prophages

P1 prophage	Relative β -galactosidase expression by λ <i>ref::lacZ</i> prophage ^a	Bof ⁺ /Bof ⁻ ratio ^b
None	100	
P1 <i>lxc-11</i> CmO	58 $\pm$ 11	0.32 $\pm$ 0.06
P1 <i>bof-1</i> CmO	56 $\pm$ 9	0.33 $\pm$ 0.13
P1 <i>cl.100</i> CmO	93 $\pm$ 24	
P1 <i>c</i> ⁺ CmO	17 $\pm$ 7	

^a Strain TSS301 was lysogenized with P1 phages. β -Galactosidase levels were measured in exponential-phase 32°C cultures as described in Materials and Methods. Measurements were made on five different sets of lysogens of strain TSS301, usually in duplicate. Enzyme incubations were usually performed for two different lengths of time; there was no systematic variation with duration of incubation. The relative expression was equal to the β -galactosidase activity (in Miller units [20]) normalized by using the activity for no P1 prophage (about 300 Miller units) and multiplied by 100. The relative activity for strain MPH30 without λ or P1 prophage was 1. The data are averages $\pm$ standard deviations for 14 determinations.

^b The ratios of *ref::lacZ* expression in the presence of Bof (supplied by P1 *c*⁺) to *ref::lacZ* expression in the absence of Bof (*lxc-11* or *bof-1*) were determined for each set of lysogens, and averages and standard deviations were calculated.

C1 synthesis was indeed elevated in *bof* mutants. There appeared to be little autoregulation in P1 *cl.100* prophages.

Plasmid-encoded *cl* genes directly repressed *cl::lacZ* transcription (Table 8), confirming previous evidence that *cl* is autoregulated (21). Autorepression by *pc1* Δ r, which encoded only P-Op99a *cl*, was about three times as effective as autorepression by pMV1w, which included most of the *immC* region (P-Op99d *orf-2 orf-3 orf-4* Op99c P-Op99a *cl*) (Table 8). In both cases, addition of plasmid-encoded Bof further repressed *cl::lacZ* expression (Table 8).

The P1 *virC* mutation, which makes the synthesis of one or more small proteins constitutive, maps just upstream from the *orf-2,3,4* region (10). The *coi* mutation, which suppresses *virC*, maps in the *orf-2,3,4* region (25). On the basis of these data, it has been proposed that *Coi* is a product of one of these

TABLE 6. Regulation of λ *ref::lacZ* prophage expression by plasmid-encoded P1 genes

C1 source	Bof source	Coi source	Relative β -galactosidase expression ^a	Ratios	
				Bof ⁺ /Bof ⁻ ^b	Coi ⁺ /Coi ⁻ ^c
None ^d	None ^d	None ^d	100		
<i>pc1</i> Δ r	None ^e	None ^d	13 $\pm$ 3.5		
<i>pc1</i> Δ r	pTS874	None ^d	7.5 $\pm$ 1.6	0.61 $\pm$ 0.24	
None	pTS874	None ^f	119 $\pm$ 17		
pMV1w	None	pMV1w	68 $\pm$ 20		5.4 $\pm$ 1.4
pMV1w	pTS874	pMV1w	29 $\pm$ 15	0.45 $\pm$ 0.24	4.0 $\pm$ 2.1

^a Strain TSS301 was transformed with plasmids, and β -galactosidase levels were determined in exponential-phase cultures as described in Materials and Methods. The relative expression was equal to the β -galactosidase activity (in Miller units [20]) normalized by using the activity obtained in the absence of other factors (about 300 Miller units) and multiplied by 100. The average relative activity for strain MPH30 without any prophage was 1. Data are averages $\pm$ standard deviations for 28 determinations (six sets of transformants, duplicate samples, two lengths of time used for the enzyme assays).

^b Ratios of *bof*⁺ *ref::lacZ* expression to *bof*⁻:Tn5 *ref::lacZ* expression in the presence of the same C1 source were calculated for each of the 28 determinations and are expressed as averages $\pm$ standard deviations.

^c Ratios of pMV1w (Coi⁺) *cl::lacZ* expression to *pc1* Δ r (Coi⁰) *cl::lacZ* expression in the presence or absence of Bof were calculated for each of the 28 determinations and are expressed as averages $\pm$ standard deviations.

^d No plasmid.

^e pTS8745 (*bof*:Tn5).

^f pBR325 or pK04.

TABLE 7. Regulation of *cl::lacZ* expression by P1 prophages

P1 prophage	Relative β -galactosidase expression from λ <i>cl::lacZ</i> prophage ^a	Bof ⁺ /Bof ⁻ ratio ^b
None	100	
P1 <i>bof-1</i> CmO	91 $\pm$ 7	
P1 <i>cl.100</i> CmO	112 $\pm$ 12	
P1 <i>c</i> ⁺ CmO	61 $\pm$ 11	0.56 $\pm$ 0.12

^a Strain TSS321 was lysogenized with P1 phages, and β -galactosidase levels were determined in exponential-phase cultures as described in Materials and Methods. The relative expression was equal to the β -galactosidase activity (in Miller units [20]) normalized by using the activity for no P1 prophage (about 4,000 Miller units) and multiplied by 100. The average relative expression for strain MPH30 was 3. Values are averages $\pm$ standard deviations for 18 determinations in which we used three sets of lysogens of strain TSS321 (duplicate samples, three lengths of time used for the assays).

^b Ratios of *cl::lacZ* expression in the presence of P1 *c*⁺ to *cl::lacZ* expression in the presence of P1 *bof-1* were determined for three sets of lysogens and are expressed as averages $\pm$ standard deviations.

reading frames and an antagonist of C1-mediated repression. Plasmid pMV1w, but not *pc1* Δ r (or the *cl::lacZ*-encoding plasmid pTS327), would be expected to encode the *coi* gene. The lesser ability to repress *ref::lacZ* and *cl::lacZ* expression shown by pMV1w is consistent with the presence of a pMV1w-encoded antagonist, but might also be ascribed to other plasmid-specific effects on *cl* expression. (*pc1* Δ r and pMV1w copy numbers appeared to be approximately equal.) The presence of a *p*_{tac} promoter in pMV1w, positioned to drive the upstream open reading frames as well as *cl* itself, provided an opportunity to rule out plasmid-specific effects and perform a preliminary *cis-trans* test for *Coi* function. We measured *cl::lacZ* and *ref::lacZ* expression, first in the presence of the moderate levels of C1 and putative *Coi* protein provided by uninduced pMV1w and then in the presence of pMV1w expression induced by various concentrations of IPTG (Fig. 5). The expression of *ref::lacZ* and the expression

TABLE 8. Regulation of λ *cl::lacZ* prophage expression by plasmid-encoded P1 genes

Source of C1	Source of Bof	Source of Coi	Relative β -galactosidase expression ^a	Ratios	
				Bof ⁺ /Bof ⁻ ^b	Coi ⁺ /Coi ⁻ ^c
None ^d	None	None	100		
<i>pc1</i> Δ r	None ^e	None	14 $\pm$ 7		
<i>pc1</i> Δ r	pTS874	None	6 $\pm$ 4	0.43 $\pm$ 0.13	
None	pTS874	None	101 $\pm$ 9		
pMV1w	None ^f	pMV1w	72 $\pm$ 10		6.4 $\pm$ 2.0
pMV1w	pTS874	pMV1w	39 $\pm$ 9	0.54 $\pm$ 0.11	9.7 $\pm$ 5.4

^a Strain TSS321 was transformed with plasmids, and β -galactosidase levels were determined in exponential-phase cultures as described in Materials and Methods. The relative expression was equal to the β -galactosidase activity (in Miller units [20]) normalized by using the activity for no P1 prophage (about 4,000 Miller units) and multiplied by 100. The average relative activity for strain MPH30 without any prophage was 0.4. The data are averages $\pm$ standard deviations for 14 determinations (five sets of transformants, multiple samples and time points).

^b Ratios of *bof*⁺ *cl::lacZ* expression to *bof*⁻:Tn5 *ref::lacZ* expression in the presence of the same C1 source were calculated for each of the 14 determinations and are expressed as averages $\pm$ standard deviations.

^c Ratios of pMV1w (Coi⁺) *cl::lacZ* expression to *pc1* Δ r (Coi⁰) *cl::lacZ* expression in the presence of *bof*⁺ or *bof*⁻:Tn5 were calculated for each of the 14 determinations and are expressed as averages $\pm$ standard deviations.

^d No plasmid.

^e pBR325 or pK04.

^f pTS8745 (*bof*:Tn5).

FIG. 5. Effect of induction of Coi and additional C1 activity on C1-regulated *ref* and *c1* expression. Cultures of strain TSS301 (A) or TSS321 (B) cells, previously transformed with pTSS874 plus pMV1w (●) or with pTSS8745 plus pMV1w (○) were grown up in supplemented M9 medium containing different concentrations of IPTG and were assayed for β -galactosidase activity as described in Materials and Methods. The relative activities were normalized for the activity of parallel cultures of strain TSS301 or TSS321 cells not transformed with any plasmids and grown without IPTG. The curves shown are representative. In other experiments the zero-IPTG levels were higher or lower and/or the Bof effects were greater or smaller, but the IPTG effects were quite similar.

of *c1::lacZ* were both markedly increased by induction of putative Coi function. Since induction would be expected to concomitantly increase synthesis of the C1 repressor, the data suggest that Coi acts *in trans* to inhibit C1 activity.

DISCUSSION

The inference drawn from previous studies (36) with P1 *bof(lxc)* and *c1(Ts)* mutants, that C1 and Bof are coregulators of *ref* gene expression, was supported by our results in experiments with isolated *c1* and *bof* genes. We demonstrated Bof-plus-C1-mediated repression of both Ref-stimulated recombination and *ref::lacZ* transcription. How might Bof act with C1 to regulate *ref*? Since multiple copies of the *bof* gene appear to have little or no regulatory effect alone, Bof seems not to act in this case as an independent repressor. Bof could in principle decrease *ref* transcription indirectly, by causing an increase in C1 levels. However, we found that Bof activity actually represses *c1::lacZ* transcription (in the presence of C1), and Schuster and co-workers (unpublished data) have found that Bof depresses the levels of immunoassayable C1. Bof might modify the C1 protein to a C1* form with increased affinity for operators or form a C1 · Bof complex with similarly altered affinity. These experiments do not distinguish between these alternatives, but it should be possible to do so by using appropriate *in vitro* assays. Bof-mediated alteration of C1 susceptibility to antagonism by the *ant(rebA, rebB)* product(s) has been proposed previously (33), but the results of our experiments with isolated *bof* genes show that this cannot be the universal mechanism for Bof action.

The observation that C1 itself and (indirectly) Bof are coregulators of *c1::lacZ* transcription may point the way to a resolution of the paradox posed by the *bof-1* mutation, which

negatively affects Ban activity but positively affects *ref* (and *ssb*) gene expression. Thus, levels of C1 regulated by Bof plus C1 might be high enough to suppress *ban* gene expression in wild-type prophages but not *bac-1* prophages, but in the absence of Bof, C1 levels might increase to the point that the *ban* gene is repressed in *bac-1* prophages as well. Thus, it appears likely that C1 tightly represses the *ban* gene without direct participation by Bof. However, the data in Tables 6 and 8 do not rule out small positive effects by Bof in the absence of C1. A final determination of the mechanisms by which Bof affects *ban* gene expression will require studies involving *ban::lacZ* and (*bac-1*)*ban::lacZ* fusions.

These data do not provide an obvious explanation for the ability of P1 *cl.100 bof-1* prophages to be induced at lower temperatures than P1 *cl.100* prophages (33), since *bof-1* prophages are expected to make more, not less, repressor. Nor is there an apparent biological rationale for enhancement of C1-mediated repression of some genes by Bof, at the same time that Bof causes C1 synthesis to decrease. The latter paradox may be related to a need to fine-tune C1 levels, so that lytic genes are tightly repressed (with the help of Bof), but genes that contribute to plasmid maintenance (in at least some P1 hosts) are only partially repressed.

The evidence that *bof* translation initiates with a TTG codon is compelling but circumstantial, and remains to be confirmed by N-terminal analysis of purified Bof protein. The putative 82-amino-acid polypeptide (Fig. 3) would have an unusual secondary structure. The high content of basic amino acids corresponds to a pI of 10.76. The hydrophobic residues are roughly grouped into four domains of 11 or so units each, separated by a few neutral residues. Many of the hydrophobic amino acids are contained in a 10-residue C-terminal tail.

In contrast to Bof, which acts with C1 as a corepressor, some activity expressed by plasmid pMV1w (but not by *pc1Δr*) appears to interfere with C1 repressor activity. It seems logical to associate this apparent C1-antagonizing activity with the Coi protein(s), whose constitutive synthesis is thought to be responsible for the phenotype of *virC* mutants (25). The *virC*-suppressing *coi* mutation maps in the *orf-2,3,4* region (25), and Baumstark et al. (B. R. Baumstark, S. R. Stovall, and P. Bralley, unpublished data) have obtained evidence that Coi is the product of *orf-4*, which is encoded by pMV1w. At moderate levels of C1 and Coi (intermediate IPTG concentrations in Fig. 5), Coi seems to have the upper hand over C1. At high IPTG concentrations, it appears that enough C1 is made to establish repression of Coi synthesis, restoring C1-mediated repression of other genes, but we cannot rule out some competitive inhibition of β -galactosidase by residual IPTG. The DNA sequence of *Orf-4*, the putative Coi protein, corresponds to a highly acidic pI of 3.68 (7). We propose that the auxiliary proteins, Bof and Coi, act by binding to C1. A C1 · Bof complex would have a significantly more positive charge than C1 alone and might be expected to bind more tightly to DNA. Conversely, a C1 · Coi complex would be more negatively charged and thus bind to DNA less well. This would explain the apparent opposite effects of Coi and Bof on expression of C1-controlled genes. Since Bof can enhance C1-mediated repression in the presence of Coi, the two proteins may compete for the same site on C1 or bind to one another. Some genes (for example, *ban*) appear to be repressed tightly by C1 alone. This model can readily be tested by biochemical experiments with purified C1, Bof, and Coi proteins.

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