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Bacteriophage P1 Bof Protein is an Indirect Positive Effector of Transcription of the Phage *bac-1 ban* Gene in Some Circumstances and a Direct Negative Effector in Other Circumstances†

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Previous genetic studies have suggested that the Bof protein of bacteriophage P1 can act as both a negative and a positive regulator of phage gene expression: in *bof-1* prophages, the *ref* gene and a putative phage *ssb* gene are derepressed, but expression of an operator-semiconstitutive variant of the phage *ban* gene (*bac-1*) is markedly reduced. An explanation of this apparent duality is suggested by recent reports that Bof is a corepressor of genes that are regulated by the phage C1 repressor, including the autoregulated *c1* gene itself. Here we show, by means of operon fusions to *lacZ*, that the balance points between Bof-mediated decreases in *c1* expression and Bof-mediated increases in C1 efficacy are different among various C1-regulated genes. Thus, expression of Bof by P1 prophages affects some genes (e.g., *bac-1 ban*) positively, and others (*e.g.*, *ref*) negatively. Even at *bac-1 ban*, where the positive indirect effect of Bof is physiologically dominant, Bof can be seen to act as a corepressor if C1 is supplied from a nonautoregulated (p_{tac}-*c1*) source, eliminating the effect of Bof on C1 synthesis.

Prophage P1 is a unit copy extrachromosomal plasmid (12). Stable lysogeny requires repression of many widely dispersed P1 lytic-gene operators by the bacteriophage C1 repressor. There are at least 14 C1 operators, numbered on the basis of their approximate map locations, e.g., Op2a, Op21, and Op51. The asymmetric operator sequences (consensus ATTGCTCTAATAAATTT) (2, 3, 5, 6) are almost always oriented in the same direction relative to associated promoters, but operator positions relative to −10 and −35 sequences vary from promoter to promoter.

Prophage P1 encodes an analog of *Escherichia coli* replication protein DnaB (4, 17): the product of the phage *ban* (DnaB analog) gene complements the Dna$^-$ phenotype of *E. coli dnaB*(Ts) mutants. P1 *bac-1* (*ban* control) mutations result in constitutive expression of the *ban* gene by P1 prophages (4). Genetic evidence (1) and DNA sequence analysis (14, 22) have demonstrated that the P1 *ban* gene is transcribed from a C1-controlled promoter, p_{ban}, associated with Op72. This operator is unique for two reasons (9). (i) It consists of two partially overlapping operators, Op72a and Op72b (see Fig. 1), arranged so as to create a site that is more nearly palindromic than most other C1 operators. (ii) Op72a is the only operator found to match every base of the C1 consensus sequence. The *bac-1* mutation is the result of a single base change in Op72a (22).

Second-site mutations which abolished the ability of P1 *bac-1* lysogens to complement *dnaB*(Ts) mutations were designated *bof* (Ban on function) (21); *bof* mutations mapped to P1 coordinate 9.5 (21). Because Ban levels appeared to be reduced in the absence of Bof, it was suggested that Bof was a positive effector of *ban* expression (21).

Subsequently however, Bof was found to be a negative regulator of the P1 *ref* gene (19). Ref activity, which enhances homologous recombination in *E. coli*, was found to be very low in wild-type prophages (24) but high in *bof-1* prophages. Furthermore, at least one additional P1 gene appeared to be negatively regulated by Bof: P1 *bof-1* prophages, unlike wild-type prophages, complemented *E. coli ssb*(Ts) mutations at nonpermissive temperatures (24). Thus arose the paradox: Bof appeared to be a positive effector of *ban* expression but a negative regulator of *ref* (and P1 *ssb*) expression.

A quantitative understanding of the role of Bof in regulation of *ref* and autoregulation of the *c1* repressor gene has emerged from recent studies with corresponding operon fusions (19). P1 *bof*$^+$ prophages repressed *ref*::*lacZ* expression and *c1*::*lacZ* expression better than did P1 *bof-1* prophages. Similarly, multicopy *c1*-encoding plasmids in *trans* partially repressed both *ref*::*lacZ* and *c1*::*lacZ* but addition of a compatible multicopy *bof* plasmid resulted in more complete repression of both fusion genes. Multicopy *bof* plasmids had very little effect in the absence of C1. These results thus showed that Bof is a negative effector of transcription of the *ref* and (autoregulated) *c1* genes. Velleman et al. (23) further implicated Bof in *c1* regulation by demonstrating that a *bof*-encoding plasmid caused a decrease in the amount of immunoassayable C1 protein expressed from a *c1*-encoding plasmid.

This role of Bof protein in autoregulation of the C1 repressor (19) suggests a resolution of the *bof* paradox. Although C1 repressor levels normally appear insufficient to repress *bac-1 ban* gene expression tightly, the elevated amount of C1 repressor expected in *bof* prophages might cause Ban activity to fall below the level required to complement *dnaB*(Ts) mutations. Thus, Bof would be an indirect

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TABLE 1. Bacterial strains, plasmids, and bacteriophages used

Strain, bacteriophage, or plasmid	Genotype or remarks	Source or reference
Strains		
MM2838	Δ(*lac-proAB*)*thi supE*	7
TSS341	MM2838 (λ341)	This work
TSS351	MM2838 (λ351)	This work
Bacteriophages		
λRS88	*'bla 'lacZ lacY lacA imm*^{434} *ind*$^-$	R. W. Simons 20
λ341	*'bla Tl*$_4$ *bac*$^+$ *ban::lacZ lacY lacA imm*^{434} *ind*$^{-a}$	This work
λ351	*'bla Tl*$_4$ *bac-1 ban::lacZ lacY lacA imm*^{434} *ind*$^{-a}$	This work
P1 CmO*r*$^-$*m*$^-$	Lacks P1 restriction, modification	M. Gottesman
P1 CmO*bof-1*	Derepressed for *ref* and for putative *ssb*	N. Sternberg
Plasmids		
pRS415	Encodes *bla Tl*$_4$ (no promoter) *'lac ZYA*	20
pSS2	Phage P1 *Eco*RI-*Sph*I fragment encoding $p_{bac^+\ ban}$ Op72, inserted into pBR325	10
pSS2-1	Same as pSS2, but $p_{bac\text{-}1\ ban}$ Op72	10
pTS341	315-bp *Hin*cII fragment encoding $p_{bac^+\ ban}$ Op72 from pSS2, inserted into unique *Sma*I site of pRS415[b]	This work
pTS351	315-bp *Hin*cII fragment encoding $p_{bac\text{-}1\ ban}$ Op72 from pSS2-1, inserted into unique *Sma*I site of pRS415[b]	This work
pAM2b	*Pvu*II-*Bcl*I subfragment of P1 *Eco*RI fragment 7 inserted into *Dra*I-restricted pKT101 (see reference 3 for details); encodes Op99e Op99d p_{coi} Op99c Op99a p_{c1} c1 (Nm^r)	3
pTS874	Phage *Bam*HI-*Bgl*II fragment inserted into *Bam*HI-restricted pACYC184; encodes *bof*$^+$; compatible with ColE1-derived plasmids (Cm^r)	19
pTS8745	Same as pTS874, but *bof*::Tn*5* (Nm^r)	19
pc1Δr	Encodes Op99a p_{c1} c1 of phage P1 in plasmid pKO-4 (Ap^r)	N. Sternberg
pKO-4	*galK* promoter-cloning vector (Ap^r)	16
pTS500	*Bam*HI-*Sal*I fragment containing the c1 gene from a fragment from pMV1w (5) inserted into corresponding sites of pJF119EH (8); encodes *lacI*^{q} p_{tac} c1 (Ap^r)	This work

[a] Constructed by homologous recombination between λRS88 and plasmid pTS341 or pTS351 as described in Materials and Methods.
[b] Construction is described in Materials and Methods.

positive effector of *bac-1 ban* expression. This hypothesis assumes that p_{ban} Op72 differs from the *ref* and c1 operator-promoters to the extent that any direct Bof corepressor effect on *bac*$^+$ *ban* and *bac-1 ban* expression is not physiologically significant.

In this study, we tested this indirect-positive-effector hypothesis by using *bac*$^+$ *ban::lacZ* and *bac-1 ban::lacZ* fusion genes. Bof indeed modulated C1 levels so as to derepress *bac-1 ban* but not *bac*$^+$ *ban* transcription; in the absence of Bof, C1 levels rose to a point at which *bac-1 ban*$^+$ transcription was significantly repressed.

MATERIALS AND METHODS

Bacteria, bacteriophages, and plasmids. The bacteria, phages, and plasmids used in this study are described in Table 1. Stocks of P1 or λ bacteriophages were prepared by confluent lysis on R or S plates, respectively, and P1 lysogens were prepared by streaking turbid centers of plaques onto LB chloramphenicol (30 μg/ml) plates as described previously (19). Plasmid DNA was extracted by the boiling (11) or alkaline-lysis (15) technique. Plasmids used for DNA sequencing were subsequently purified by isopycnic sedimentation in CsCl-ethidium bromide.

Media and buffers. TBY broth, LB plates, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) plates, R plates, S plates, TCMB plates, and Z buffer were as previously described (19). Antibiotics were used at the following concentrations: ampicillin, 75 μg/ml; neomycin, 50 μg/ml; chloramphenicol, 30 μg/ml.

Recombinant DNA techniques. Restriction enzymes and T4 DNA ligase were purchased from New England BioLabs or U.S. Biochemical Corp. and used as recommended by the suppliers. Gel electrophoresis, electroelution of DNA restriction fragments, ligations, and DNA transformations were performed as previously described (19).

Construction of multicopy and single-copy operon fusions. Promoterless *lacZ* fusion vector plasmid pRS415 was restricted with endonuclease *Sma*I and ligated to 315-bp *Hin*cII fragments containing $p_{bac^+\ ban}$ Op72 or $p_{bac\text{-}1\ ban}$ Op72, previously isolated from plasmid pSS2 (*bac*$^+$ *ban*$^+$) or pSS2-1 (*bac-1 ban*). Since previous studies have shown that certain P1 promoter-operators are lethal when present on multicopy plasmids (10, 24), the ligation products were transformed into Δ*lac* bacteria (MM2838) containing either a P1 *c*$^+$ prophage or plasmid pAM2b as a C1 repressor source. Transformants forming blue colonies on (ampicillin-containing) X-Gal plates were used to isolate plasmids. Plasmids were screened by restriction analysis for inserted fragments of the appropriate size. The orientation of inserts in *bac*$^+$ *ban::lacZ* (pTS341) and *bac-1 ban::lacZ* (pTS351) plasmids was verified by direct double-stranded DNA sequencing (26) by using the M13 universal sequencing primer and Sequenase (U.S. Biochemical Corp.). Fusion genes were transferred from plasmids to λ phages by homologous recombination during plate stock growth as previously described (19) or during single-cycle lytic growth of vector phage λRS88 on MM2838 bacteria harboring pTS341 or pTS351. In the single-cycle procedure, plasmid-containing bacteria were adsorbed to λRS88 at a multiplicity of 0.1 phage per cell at 37°C for 15 min and TBY broth was added to each mixture. The cultures

were incubated for an additional 90 min at 37°C and then treated with $CHCl_3$. Lysates were plated with MM2838 bacteria on X-Gal plates. Blue plaques were purified three times and used to prepare phage stocks by confluent lysis. Stocks were used to lysogenize MM2838. Light blue lysogens were repeatedly streaked on X-Gal plates until stable. Single and multiple lysogens were distinguished by a Ter test as previously described (20). Single lysogens were used for all subsequent experiments.

Measurement of β-galactosidase. Bacteria lysogenic for fusion gene-encoding λ prophages were assayed in log-phase liquid cultures as described previously (19). Cells were diluted with 9 volumes of 0.85% NaCl prior to dilution in Z buffer. Assay mixtures were warmed to 37°C before addition of *o*-nitrophenyl-β-D-galactopyranoside substrate and then incubated at 37°C for 5 to 20 min, depending on activity levels.

RESULTS

In order to mimic expression of *ban* genes in unit copy P1 prophages, we isolated bacteria lysogenic for single λ prophages encoding *bac*$^+$ *ban*::*lacZ* or *bac-1 ban*::*lacZ* fusion genes. The respective λ phages were obtained by homologous recombination between vector phage λRS88 (Table 1) and the corresponding plasmids. The latter had been constructed by inserting 315-bp DNA fragments encoding the promoter-operator regions of P1 wild-type and *bac-1* phages (Fig. 1).

Expression of Bof activity by P1 prophages increases transcription of the *bac-1 ban* gene but decreases transcription of the *ref* and *c*1 genes. The effects of P1 *bof*$^+$ and *bof-1* prophages in *trans* on expression of *bac*$^+$ *ban*::*lacZ* and *bac-1 ban*::*lacZ* fusion genes were compared with previously reported effects on *ref*::*lacZ* and *c*1::*lacZ* expression (19) (Table 2). Three different patterns were observed.

(i) Either prophage completely blocked *bac*$^+$ *ban*::*lacZ* expression (Table 2, column 2). This suggests that the *ban* gene is repressed very tightly by C1 alone, so that Bof function has no physiologically significant direct role at p_{ban} Op72. (ii) Bof had a positive effect on *bac-1 ban*::*lacZ* expression (Table 2, column 3). As expected, the *bac-1* mutation rendered *ban* expression less sensitive to C1 repression (Table 2, column 3). Evidently the level of *ban* transcription in *bac-1 ban* prophages, seen here to be 34% of derepressed levels, suffices for complementation of *dnaB* (Ts) mutations (4); under the same conditions, *bac*$^+$ *ban* transcription appeared negligible (Table 2, column 2). In bacteria lysogenic for P1*bof-1*, the absence of the Bof contribution to *c*1 autorepression would be expected to elevate C1 levels (19, 23). In agreement with this prediction, *bac-1 ban*::*lacZ* transcription was reduced to 10% of maximal in such lysogens (Table 2, line 3). Evidently this level of expression corresponds to too little Ban protein for complementation of *dnaB*(Ts) mutations. (iii) Bof was a direct negative effector of *ref* and *c*1 transcription in the presence of C1 (Table 2, columns 4 and 5), as previously reported (19).

When *bac*$^+$ *ban*::*lacZ* expression and *bac-1 ban*::*lacZ* expression were compared with one another in parallel experiments, the fully derepressed activity of the mutant gene was only about two-thirds of that of the wild-type *ban* gene (Table 3, footnote *a*). Thus, the proposal that the *bac-1* mutation (a C→A transversion at position 5 of Op72 [Fig. 1]) might increase absolute promoter strength, by making the −35 region of the promoter more nearly resemble the *E. coli* consensus sequence (9, 14), was not supported.

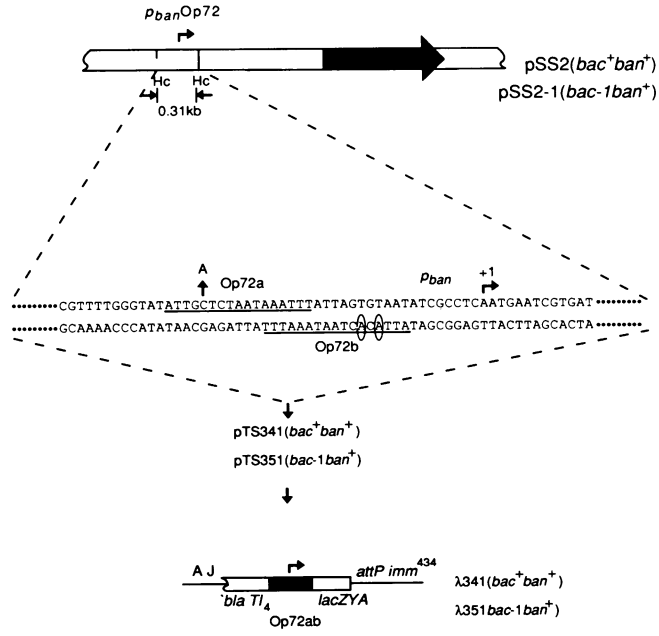

FIG. 1. Region of P1 DNA analyzed by operon fusion techniques. *Hin*cII (Hc) fragments (0.31 kb) encoding *bac*$^+$ *ban* (from pSS2) or *bac-1 ban* (from pSS2-1) promoter-operator regions (indicated by the small rightward-pointing arrow above the fragment) were ligated to *Sma*I-linearized pRS415, yielding plasmids pTS341 and pTS351, as described in Materials and Methods. The heavy rightward-pointing arrow represents the *ban* structural gene. The sequence of the p_{ban} Op72 promoter-operator region is shown (9, 22). The location and nature (22) of the single base transversion which results in the *bac-1* phenotype is indicated by an upward-pointing arrow. The underlined sequences illustrate C1 repressor-binding sites Op72a and Op72b; circled nucleotides represent deviations from the derived C1 consensus sequence (2, 3, 5, 6). The rightward-pointing small arrow (+1) represents the nucleotide at which transcription initiates in vitro (9). A representative example of a recombinant lambda phage encoding a *ban*::*lacZ* operon fusion is shown at the bottom.

Bof is not a positive regulator of expression of *bac*$^+$ *ban* or *bac-1 ban*. The prophage-in-*trans* data demonstrate that the positive effect of Bof on *bac-1 ban* expression is at the level of transcription but do not distinguish between an indirect Bof effect, via its role in C1 autoregulation, and a direct positive effect. To test the latter notion, we measured the effects on transcription of plasmid-encoded Bof, in the absence or presence of C1 repressor expressed by multicopy plasmids (Table 3). C1 repressor alone sufficed to reduce *ban*$^+$ and *bac-1 ban* expression from λ prophages by 95 to 98% (Table 3, lines 2 and 4), and addition of Bof had no significant effect. The difference between negligible expression of *bac ban*::*lacZ* in the presence of P1 prophages (Table 2) and low but measurable expression in the presence of plasmid-encoded C1 (and Bof) (Table 3) may reflect a minor regulatory role by some additional P1 element. In the absence of C1, Bof alone may slightly repress both *bac*$^+$ *ban* expression and *bac-1 ban* expression (Table 3, line 3) but the data are not precise enough for this small effect to be considered significant. It is clear, however, that Bof is not a direct positive effector of *ban* expression, either alone or in the presence of autoregulated C1.

The *bac-1 ban* promoter-operator is intrinsically susceptible to Bof-C1 corepression. Can Bof act at the *ban* promoter-

TABLE 2. Regulation of transcription of *lacZ* genes fused to phage P1 promoter-operators by P1 prophage in *trans*

P1 prophage	Avg relative β-galactosidase activity ± SD			
	λ*bac*$^+$ *ban*::*lacZ*[a]	λ*bac-1 ban*::*lacZ*[a]	λ*ref*::*lacZ*[b]	λ*c*1::*lacZ*[b]
None	100	100	100	100
P1 *c*$^+$ *bof*$^+$	<1	34 ± 9	17 ± 7	61 ± 11
P1 *c*$^+$ *bof-1*	<1	10 ± 3	56 ± 9	91 ± 7

[a] Bacteria lysogenic for single λ*bac*$^+$ *ban*::*lacZ* (TSS341) and λ*bac-1 ban*::*lacZ* (TSS351) prophages were lysogenized with P1 *c*$^+$ or P1 *c*$^+$ *bof-1*. Double lysogens were grown at 37°C to exponential phase in TBY broth supplemented with chloramphenicol. β-Galactosidase activity was measured as previously described (18). Experiments with *bac*$^+$ *ban*::*lacZ* and *bac-1 ban*::*lacZ* were performed separately. The *bac*$^+$ data represent four independent experiments (a total of 26 determinations). The *bac-1* data represent five independent experiments (a total of 28 determinations). Relative expression equals β-galactosidase activity divided by activity in the absence of a P1 prophage multiplied by 100. A relative activity of 100 corresponds to 11,000 ± 1,800 (*bac*$^+$ *ban*), 8,600 ± 1,700 (*bac-1 ban*), 300 (*ref*), or 4,000 (*c*1) Miller units.

[b] These data are from previously described similar experiments with λ*ref*::*lacZ* and λ*c*1::*lacZ* prophages (19) and are included for comparison.

operator as a corepressor with C1 under any circumstances, or is *ban* intrinsically different from the *ref* and *c*1 promoter-operators in this respect? By the former hypothesis, potential Bof-C1 corepression at *ban*, masked by the effect of Bof on C1 autoregulation, would be revealed if C1 were supplied from a nonautoregulated source. By the latter, transcription initiation at the *bac*$^+$ *ban* and *bac-1 ban* promoter-operators would be indifferent to Bof, even if C1 levels were not autoregulated. To test these hypotheses, we placed the *c*1 structural gene under control of the p_{tac} promoter, so that C1 levels were controlled by the *lac* repressor and, therefore, inducible by isopropyl-β-D-thiogalactopyranoside (IPTG). The wild-type *ban* promoter has such a high affinity for C1 that *bac*$^+$ *ban*::*lacZ* expression was completely (>99%) repressed, even in the absence of IPTG, in the presence or absence of Bof (Fig. 2, upper panel). It was thus not possible to test the hypotheses in this case. However, *bac-1 ban*::*lacZ* expression was at 11% of derepressed levels in the absence of IPTG; it was further repressed (nearly 10-fold) by

TABLE 3. Regulation of expression of *bac*$^+$ *ban*::*lacZ* and *bac-1 ban*::*lacZ* fusion genes by plasmid-encoded Bof and C1 activities[a]

C1 source	Bof source	Avg relative β-galactosidase activity ± SD[b]	
		λ*bac*$^+$ *ban*::*lacZ*	λ*bac-1 ban*::*lacZ*
None	None	100	100
pc1Δr	None[c]	2.4 ± 2.4	4.8 ± 2.1
None[d]	pTS874	77 ± 22	82 ± 16
pc1Δr	pTS874	3.5 ± 2.4	3.8 ± 3.5

[a] Bacteria lysogenic for single λ*bac*$^+$ *ban*::*lacZ* (TSS341) or λ*bac-1 ban*::*lacZ* (TSS351) prophages were transformed with the indicated plasmids. Transformants were grown to exponential phase in TBY broth supplemented with ampicillin, chloramphenicol, and kanamycin (for selection of pTS8745) at 37°C. β-Galactosidase activity was measured as previously described (19). The data represent six independent experiments (a total of 32 determinations). Experiments with both fusions were performed simultaneously: relative expression equals β-galactosidase activity for the indicated constructs divided by activity for TSS341 or TSS351 containing no plasmids, multiplied by 100.

[b] Absolute expression of β-galactosidase from λ351 (*bac-1 ban*::*lacZ*) prophages was 0.70 ± 0.13 times the expression from λ341 (*bac*$^+$ *ban*::*lacZ*) prophages in the absence of C1 and Bof sources.

[c] Plasmid pTS8745.

[d] Plasmid pKO-4.

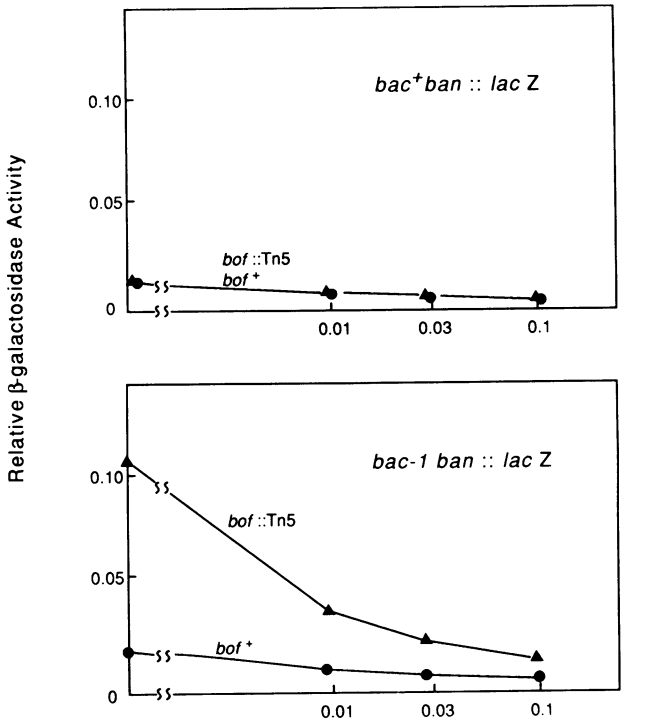

FIG. 2. Regulation of *bac*$^+$ *ban* and *bac-1 ban* transcription by nonautoregulated C1 plus Bof. Bacteria lysogenic for single λ*bac*$^+$ *ban*::*lacZ* (TSS341) and λ*bac-1 ban*::*lacZ* (TSS351) prophages and harboring p_{tac}-*c*1 plasmid pTS500 were transformed with plasmids encoding *bof*$^+$ (pTS874) (●) and *bof*::Tn*5* (pTS8745) (▲). Single transformed colonies were suspended in 0.85% NaCl. Aliquots were transferred to TBY broth with ampicillin, chloramphenicol, and neomycin (for selection of pTS8745) containing 0, 10, 30, or 100 μM IPTG. Cultures were grown to mid-log phase at 37°C and assayed for β-galactosidase activity as previously described (19). The data shown are averages of quadruplicate determinations. Relative expression equals β-galactosidase activity divided by activity expressed by bacteria lysogenic for λTSS351, but containing neither *c*1- nor *bof*-encoding plasmids, multiplied by 100. The data presented are representative of similar experiments performed on different days. The effect of Bof observed in other experiments was slightly greater or slightly less than that shown here.

induction of additional C1 synthesis. At every level of C1 induction, the presence of Bof activity caused a further decrease (three- to seven-fold) in *bac-1 ban*::*lacZ* expression (Fig. 2, lower panel). Thus, *bac-1 ban*, and perhaps *bac*$^+$ *ban* by extension, can be seen to be intrinsically susceptible to Bof-C1 corepression if the masking effect of Bof involvement in C1 autoregulation is removed and if C1 levels are low enough that there is room for a Bof effect.

DISCUSSION

The genomic organization of bacteriophage P1 is quite different from that of λ, the temperate-phage paradigm. In contrast to the operators associated with the two divergent master promoters in lambdoid phages, the P1 operators are widely separated; the phage functions that they control are numerous and varied. P1 operator-promoters appear to differ widely among themselves with respect to affinity for the C1 repressor and dependence on the Bof corepressor for max-

imum down-regulation of transcription. Maximum repression by C1 of its own synthesis requires Bof; this provides a mechanism for further fine tuning of regulatory circuits. Since prophage P1 is a plasmid, rather than an integral part of the bacterial chromosome, as the lambdoid prophages are, it seems likely that expression of some P1 genes must be delicately adjusted to levels intermediate between those corresponding to wide-open lytic growth and total shutdown. Recent studies have provided some clues as to how this fine tuning might be accomplished.

The *bac*$^+$ *ban* and mutant (*bac-1 ban*) promoter-operators investigated here increase to four the number of P1 transcription-regulation elements studied quantitatively by operon fusion techniques. These two genes provide good examples of the possibilities for subtle adjustment of P1 gene expression. Here we have shown that (i) the inability of P1 *bac-1 bof-1* prophages to complement *E. coli dnaB*(Ts) mutations is most likely a direct result of reduced initiation of transcription at the *bac-1 ban* promoter-operator, (ii) Bof is not a positive effector of *bac-1 ban* transcription, and (iii) at least *bac-1 ban* is intrinsically susceptible to Bof-C1 corepression once the physiologically dominant effect of Bof on C1 autoregulation is removed.

The *bac*$^+$ *ban* promoter-operator p_{ban} Op72 differs from those of other genes studied by operon fusion techniques (*bac-1 ban*, *ref*, and *c*1) in that *bac*$^+$ *ban* expression is completely repressed by low levels of C1 alone; thus, Bof, a corepressor of several other C1-regulated genes, is irrelevant to regulation of *bac*$^+$ *ban*. Even the presumably low levels of C1 expressed by *lacI*^{q} p_{tac}-*c*1 plasmids in the absence of IPTG repress *bac*$^+$ *ban*::*lacZ* expression by more than 99%, despite the high intrinsic strength of p_{ban}. We do not know whether or not Bof would act as a corepressor at p_{ban} Op72 at even lower in vivo C1 concentrations, but recent in vitro work suggests that this is the case. Velleman et al. (23) showed that purified Bof protein slightly enhanced the ability of low levels of purified C1 protein to retard a *bac*$^+$ *ban* promoter-operator DNA fragment during electrophoresis; even lower C1 levels might well have revealed a greater Bof effect.

What might account for the high affinity of Op72 for C1 protein? Two aspects of the *ban* promoter-operator architecture may be significant here. (i) Its two tandem overlapping C1-binding sites distinguish it from single C1 site promoter-operators, such as p_{ref} Op2a (25); the two p_{ban} Op72 sites are designated Op72a and Op72b. (ii) Although some other P1 promoter-operators [e.g., Op99a(b)] (23) display this overlapping-tandem C1 site motif, Op72a is the only P1 operator to provide a perfect match to the consensus C1 site (the match is 15 of 17 for Op72b). In contrast, the matches are 15 of 17 for Op99a and only 11 of 17 for the quasi-site that overlaps Op99a. These differences may be sufficient to account for the observations that even very low concentrations of C1 suffice for full repression at Op72 without corepression by Bof, whereas full repression at Op99a requires Bof (19), even when C1 concentrations are very high (data not shown).

The *bac-1* mutation, a single base change in Op72a, reduces the intrinsic promoter strength of $p_{bac\text{-}1\ ban}$ to 70% of that of $p_{bac^+\ ban}$. However, the mutation simultaneously reduces the affinity of Op72 for C1 to the point at which, even in the presence of a P1 prophage, *bac-1 ban*::*lacZ* expression is 0.34 times the derepressed level (compared with <0.01 times for *bac*$^+$ *ban*::*lacZ*). Thus, in *bac-1 ban* lysogens the concentration of *ban* transcripts would be expected to be at 24% of the concentration corresponding to a fully derepressed *ban*$^+$ gene (0.34 × 70%). This would account for the ability of the mutant prophages to complement *E. coli dnaB*(Ts) mutations. C1 still regulates the mutant operator, however; *bac-1 ban*::*lacZ* transcription is repressed 67 and 90% by (single-copy) wild-type and *bof-1* prophages, respectively, 95% by a multicopy *c*1-encoding plasmid, and over 98% by an induced p_{tac}-*c*1 plasmid. The sensitivity of *bac-1 ban* expression to C1 levels fortuitously set the stage for discovery of the *bof* gene (21). In a prophage, the *bof-1* mutation causes steady-state C1 levels to increase to the point at which *bac-1 ban*::*lacZ* expression in *trans* is reduced to 0.10 times the derepressed value. This would correspond to *ban* transcripts in *bac-1 bof-1* lysogens at only 7% of derepressed *ban*$^+$ levels, evidently not enough to complement *E. coli dnaB*(Ts) mutations.

Thus, the window between *dnaB*(Ts) complementation at 24% *ban* expression and lack of complementation at 7% *ban* expression made it possible to isolate the *bof-1* mutation on the basis of its apparent Ban$^-$ phenotype. Although Bof was originally proposed to be a positive effector of *ban* expression, the Ban$^-$ phenotype of *bof* mutants seems to be entirely a result of the role of Bof in C1 autoregulation. Bof is not a positive effector of expression of either *bac*$^+$ *ban*::*lacZ* or *bac-1 ban*::*lacZ*, in the presence or absence of C1. In fact, in the presence of nonautoregulated C1, Bof can be seen to be a direct negative regulator of *bac-1 ban*::*lacZ* expression, as it is of *ref* and *c*1 expression. Neither this direct negative Bof effect at *bac-1 ban* nor a possible direct negative effect at *bac*$^+$ *ban* seems to be physiologically important, being masked by the role of Bof in C1 autoregulation.

Thus, the original basis for the *bof* acronym, Ban on function, appears to be a misnomer. Since P1 *lxc* mutations, which confer constitutive expression of *ref* and a putative *ssb* gene, appear to be allelic with *bof* (24), it has been suggested that *lxc* be reinterpreted as lowers expression of *c*1 (23). [The *lxc* designation originated by way of analogy with *E. coli lexC* mutations, on the basis of the ability of P1 *lxc* prophages to suppress *E. coli ssb*(Ts) mutations (13).] However, Bof down-regulates expression of genes other than *c*1 (19), so a more general acronym seems appropriate. We suggest that the designation *bof* be retained but reinterpreted as bolsters C-one function. This would be consistent with the corepressor activity of Bof seen at most of the P1 promoter-operators tested thus far and with the apparent absence of significant Bof function in the absence of C1 repressor.

The dozen or more P1 genes controlled by the phage primary (C1) repressor seem capable of being expressed simultaneously at quite different levels. The Bof protein modulates C1-mediated repression by acting as a corepressor, thus exerting a direct negative effect on expression of some genes; Bof simultaneously down regulates C1 levels, thus exerting an indirect positive effect on other genes. It will be of interest to elucidate the role of this versatile fine-tuning element in regulation of other C1-controlled genes.

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