JOURNAL OF BACTERIOLOGY, Mar. 1988, p. 1092–1102
0021-9193/88/031092-11$02.00/0
Copyright © 1988, American Society for Microbiology
Vol. 170, No. 3

Control of Bacterial Alkaline Phosphatase Synthesis and Variation in an *Escherichia coli* K-12 *phoR* Mutant by Adenyl Cyclase, the Cyclic AMP Receptor Protein, and the *phoM* Operon

BARRY L. WANNER,* MARY R. WILMES, AND DENNIS C. YOUNG
Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

Received 15 December 1986/Accepted 3 December 1987

Mutant *phoR* cells show a clonal variation phenotype with respect to bacterial alkaline phosphatase (BAP) synthesis. BAP clonal variation is characterized by an alternation between a Bap$^+$ and Bap$^-$ phenotype. The switching is regulated by the *phoM* operon and the presence of glucose; the *pho-510* mutant form of the *phoM* operon abolishes both BAP clonal variation and the effect of glucose (B. L. Wanner, J. Bacteriol. 169:900–903, 1987). In this paper we show that a mutation of the adenyl cyclase (*cya*) and the cyclic AMP receptor protein (*crp*) gene also abolish BAP clonal variation; either simultaneously reduces the amount of BAP made in *phoR* mutants. Also, the *pho-510* mutation is epistatic; it increases BAP synthesis in Δ*cya phoR* and Δ*crp phoR* mutants. These data are consistent with the wild-type *phoM* operon having a negative, as well as a positive, regulatory role in gene expression. Furthermore, the data suggest that adenyl cyclase and Crp indirectly regulate BAP synthesis in a *phoR* mutant via an interaction with the *phoM* operon or its gene products. However, *phoM* operon expression was unaffected when tested with *phoM* operon *lacZ* transcriptional fusions. In addition, the switching Bap phenotype was not associated with an alternation in *phoM* operon expression.

Bacterial alkaline phosphatase (BAP) synthesis in *phoR* mutants shows an alternating pattern of gene expression (20, 31) which is phenotypically similar to classic *Salmonella* phase variation (17). The switching is called BAP clonal variation; it acts at the transcriptional level of the BAP structural gene, *phoA*, and is *recA* independent. The alternation in *phoA* gene transcription is regulated in *trans* by the unlinked *phoM* operon; the variation is also affected by the growth medium, especially by the presence of glucose (21).

The BAP clonal variation phenotype of *phoR* mutants was discovered only recently, even though the same *phoR* mutations had been studied for over 25 years (20, 31). This discovery was made when we cloned the wild-type *phoM* operon and noticed that our multicopy plasmids led to a metastable Bap phenotype when transformed into Bap$^-$ Δ*phoM phoR* mutants (24, 31). That is, our transformants showed a switching Bap phenotype; they alternately expressed a Bap$^+$ and Bap$^-$ phenotype (31). This observation led to the discovery that some *phoR* mutant strains showed a similar metastable behavior, whereas other *phoR* mutants did not (20). Further studies revealed that the phenotype of *phoR* mutants depended upon the strain background; it was independent of the *phoR* allele (20). A genetic difference in the *phoM* operon among common laboratory *Escherichia coli* K-12 strains, the *pho-510* mutation, was responsible for constitutivity in some *phoR* strains (20, 21, 31; this paper). The *pho-510* mutation apparently arose after X-ray mutagenesis over 40 years ago in the formation of strain 58F$^+$, an ancestral *E. coli* K-12 strain (3, 21). As a consequence, the *pho-510* mutation is now widespread; it exists in descendants of strain 58F$^+$, including several popular Hfr strains such as HfrC and HfrH (21). The *pho-510* mutation existed also in several strains that had been previously used to study *phoA* gene regulation (21, 23).

The *phoM* gene was discovered because mutations in it abolish BAP synthesis in a constitutive *phoR pho-510* mutant (20, 27). The DNA sequencing of the *phoM* region showed an operon with four open reading frames (ORFs) (1). ORF3 corresponds to the *phoM* gene, which is required for BAP synthesis only in *phoR* mutants (20, 23, 27). In this paper, the term *phoM* operon will refer to the entire operon containing the *phoM* gene, whereas *phoM* gene or PhoM will refer only to the DNA or protein corresponding to ORF3. Different allelic forms of the *phoM* operon show either of three Bap phenotypes in *phoR* mutants: (i) the wild-type *phoM* operon leads to the metastable clonal variation phenotype, in which BAP synthesis also responds to environmental catabolites such as glucose; (ii) the *pho-510* mutant form leads to constitutivity; and (iii) the doubly mutated forms, such as the *pho-510 phoM451*(Am) operon, as well as Δ*phoM* mutations lead to a Bap$^-$ phenotype. However, the *phoM* operon has no effect on BAP synthesis in *phoR*$^+$ bacteria; even deletions of the entire *phoM* operon are without effect (24).

Interestingly, the ORF2 and *phoM* (ORF3) genes in the *phoM* operon share similarities at the protein sequence level with several two-component environmental regulatory systems (16; C. Ronson, personal communication). Therefore genes in the *phoM* operon may have a regulatory function (31), in addition to a role in controlling transcription of *phoA* and other genes of the phosphate regulon in *phoR* mutants (23). Based upon the protein sequence similarities, the ORF2 and *phoM* (ORF3) gene products in the *phoM* operon are similar to the *phoB* and *phoR* gene products, which regulate *phoA* transcription in response to phosphate limitation (23). The ORF2 and *phoB* proteins are similar to DNA binding proteins that are activators; the *phoM* (ORF3) and *phoR* proteins are similar to environmental sensory or transducer proteins (16). The *pho-510* mutation that abolishes BAP clonal variation in *phoR* mutants maps in ORF1 or ORF2 of the *phoM* operon (31; this paper).

The protein sequence similarities between PhoR and

* Corresponding author.

TABLE 1. Bacterial strains

Strain	Genotype[a]	Pedigree	Construction or reference
BW2091	DE93 (*phoM dye thr chlG*) Δ*lac-169 rpsL267 crp-72 thi*	XPh1a	24
BW8661	*phoM451*(Am) *pho-510 phoR68* Δ*lac-169 rpsL267 crp-72 thi*	XPh1a via BW2091	Thr$^+$ with P1 grown on BW499 (27)
BW8877	Δ*cya-6* DE93(*phoM dye thr chlG*) *phoR68* Δ*lac-169 rpsL267 crp-72 thi*	XPh1a via BW8622	Ilv$^+$ with P1 grown on CA8439 (4)
BW8881	*phoM*(wt) *phoR68* Δ*lac-169 rpsL267 crp-72 thi*	XPh1a via BW2091	Thr$^+$ with P1 grown on BW3627 (20)
BW8882	*pho-510 phoR68* Δ*lac-169 rpsL267 crp-72 thi*	XPh1a via BW2091	Thr$^+$ with P1 grown on BW3912 (19)
BW8898	Δ*crp-39* DE93(*phoM dye thr chlG*) *phoR68* Δ*lac-169 rpsL267 thi*	XPh1a via BW8887	Aro$^+$ with P1 grown on CA8439
BW9082	Δ*cya-6 pho-510 phoR68* Δ*lac-169 rpsL267 crp-72 thi*	XPh1a via BW8877	Thr$^+$ with P1 grown on BW3912
BW9083	Δ*cya-6 phoM*(wt) *phoR68* Δ*lac-169 rpsL267 crp-72 thi*	XPh1a via BW8877	Thr$^+$ with P1 grown on BW3627
BW9084	Δ*cya-6 phoM453 pho-510 phoR68* Δ*lac-169 rpsL267 crp-72 thi*	XPh1a via BW8877	Thr$^+$ with P1 grown on BW304 (27)
BW9085	Δ*cya-6 phoM451*(Am) *pho-510 phoR68* Δ*lac-169 rpsL267 crp-72 thi*	XPh1a via BW8877	Thr$^+$ with P1 grown on BW499
BW9593	*pho-510 phoM*::*lacZ*(Mu d1-8)$_1$ *phoR68* Δ*lac-169 srlC300*::Tn*10 recA1*	BD792 via BW9561	Tc^r with P1 grown on BW9355 (*srlC300*::Tn*10 recA1*/λ*recA*$^+$) (31)
BW9594	Δ*cya-6 pho-510 phoM*::*lacZ*(Mu d1-8)$_1$ *phoR68* Δ*lac-169 srlC300*::Tn*10 recA1*	BD792 via BW9562	Tc^r with P1 grown on BW9355
BW9596	*pho-510 phoM*::*lacZ*(Mu d1-8)$_2$ *phoR68* Δ*lac-169 srlC300*::Tn*10 recA1*	BD792 via BW9564	Tc^r with P1 grown on BW9355
BW9836	*zjj-101*::*cat*R62R12 *pho-510 phoM*::*lacZ*(Mu d1-8)$_2$ Δ*lac-169*	BD792 via BW9090	Cm^r with P1 grown on JC7623 (R101C)[b]
BW10128	*zjj-101*::*cat*R62R12 *pho-510 phoM*::*lacZ*(Mu d1-8)$_1$ *phoR68* Δ*lac-169*	BD792 via BW9320	Cm^r with P1 grown on JC7623 (R101C)
BW10129	*zjj-101*::*cat*R62R12 *pho-510 phoM*::*lacZ*(Mu d1-8)$_2$ *phoR68* Δ*lac-169*	BD792 via BW9321	Cm^r with P1 grown on JC7623 (R101C)
BW10189	*phoM*(wt) *thr*::*lacZ*[λp1(*209*)]Ts$^+$ *phoR68* Δ*lac-169 tsx-234*::Tn*10* IN(*rrnD-rrnE*)*1*	W3110 via BW10127	Tc^r with P1 grown on BW9033 (*tsx-234*::Tn*10 phoR68*)

[a] *phoM*(wt) indicates the wild-type *phoM* operon. Ts$^+$ means that temperature-resistant growth was selected.
[b] This strain has the wild-type *phoM* operon (unpublished data).

PhoM (ORF3) (16) suggest that they may act in a similar manner. Since either PhoR or PhoM (ORF3) regulates *phoB*-dependent *phoA* transcription (27), this dual control over the phosphate regulon could reveal "crosstalk" among environmentally responsive promoters (16). Interestingly, we previously showed that the expression of several phosphate-starvation-inducible (*psi*) promoters was also specifically induced by other environmental stimuli, including nitrogen and carbon limitations, UV irradiation, and anoxia (18, 28). Crosstalk could account for the molecular basis of such overlapping physiological controls in the phosphate regulon. Along this line, we also previously showed that expression of the *psiE* and *psiO*, but not the *phoA*, promoter was affected (in a *phoR*$^+$ strain) by an adenyl cyclase (*cya*) or cyclic AMP (cAMP) receptor protein (*crp*) mutation (18, 28). In this paper we show that a Δ*cya* and Δ*crp* mutation alters BAP synthesis and abolishes variation in *phoR* mutants. The role for the *phoM* operon in gene expression is also discussed.

The nomenclature *phoM*(wt) was adopted for the wild-type *phoM* operon because numerous studies were previously done in strains with the *pho-510* mutant form of the *phoM* operon (21, 31). The *phoM*(wt) designation is now used in place of the *pho-499* allele, which had been assigned to the wild-type *phoM* operon before the genetic basis of the variable Bap phenotype was traced to wild-type *E. coli* K-12 lines (19, 21, 31).

MATERIALS AND METHODS

Bacterial strains. BAP regulation was studied in derivatives of the *E. coli* K-12 strains XPh1a (4) and BD792 (18). The XPh line was mutagenized several times in the past; its construction also involved multiple Hfr crosses (4). It was used because many *pho* mutations, including all *phoM* operon alleles, exist in the XPh lineage. The parental XPh strain in this paper generally had the following genotype: Δ*lac-169 pho-510 rpsL267 crp-72 thi*, where the *crp-72* allele is a leaky mutation that was described elsewhere (28). Strain BD792 is an unmutagenized *E. coli* K-12 strain that was cured of λ and F$^+$ factor (B. Duncan, personal communication). The Δ*lac-169* mutation was crossed into it with P1 (18). Strain CA8439 (Δ*cya-6* Δ*crp-39*) (5) was obtained from J. Beckwith; strain CU1248 (*ilvY864*::Tn*10*) (9) was from H. E. Umbarger; JC7623(R101C) (*zjj-101*::*cat*) was from R. Somerville; and TT9894 [Mu *c62*(Ts$^-$) Mu d1-*8*] (10) was from J. Roth. The complete genotypes for bacteria that were assayed are given in Table 1.

Strain constructions. Unless noted otherwise, all derivative bacteria were made in a series of P1 crosses. The Δ*cya-6* mutation was introduced in two steps: the recipient was made tetracycline resistant (Tc^r) with P1 grown on CU1248; it was then made Ilv$^+$ with P1 grown on CA8439. The Δ*crp-39* allele was introduced in three steps: a *malT* mutant was selected as a spontaneous λ*vir*^{r} Mal$^-$ mutant; it was made Mal$^+$ and *aroB* with P1 grown on BW365, a W3110 strain with the *aroB* allele of strain I7010 (29; unpublished data); and an *aroB* transductant was made Aro$^+$ and Crp$^-$ with P1 grown on CA8439. Cya$^-$ or Crp$^-$ mutants were identified by their Mal$^-$ phenotype (5).

Media, chemicals, and recombinant DNA methods. Media, chemicals, and recombinant DNA methods were as described previously (19, 22, 27). XG and XP media produce a blue dye after hydrolysis by β-galactosidase and BAP (29), respectively.

Enzyme assays. β-Galactosidase and BAP activities were measured in chloroform-sodium dodecyl sulfate-lysed cells as described previously (20, 22, 23, 29). Cells were grown for assay at 37°C on TYE agar. An appropriate antibiotic was also present to maintain plasmids (31). When variants were assayed (20) for both activities, colonies were first suspended in a few tenths of a milliliter of 0.05 M Tris (pH 8.0), and portions were used to measure the cell optical density at 420 nm and enzyme activities. Units are nanomoles of product made per minute.

Genetics. The *dye* phenotype (8) was tested by streaking cells onto toluidine blue plates (6). Dye^+ cells are dye resistant (Dye^r). The *chlG* phenotypic test was reported previously (24).

Construction of *phoM* operon *lacZ* transcriptional fusions. Mu d1-8 donor lysates were prepared by heat induction of strain TT9894 (10) and used to transduce strain BW8643 (Su^+ *phoR pho-510*) to ampicillin resistance. A *pho-510 phoR* strain was used to allow identification of *phoM* mutants by their Bap^- phenotype. (In attempts to identify Bap^- mutants of a *phoR* strain with the wild-type *phoM* operon, the vast majority of Bap^- colonies proved simply to be variants expressing the Bap^- phenotype [data not shown].) Ampicillin-resistant (Amp^r) transductants were selected on tryptone-yeast extract BAP indicator (TYE-XP) agar containing 25 μg of ampicillin per ml. Nine independent Bap^- mutants were found among approximately 10,000 Amp^r transductants. Six were *thr* linked when tested as recipients with P1 grown on a *thr*::Tn*10* mutant. Four were also tested as donors and yielded Thr^+ transductants of strain BW2091 [Su^- Δ(*phoM-thr*) *phoR*] (Table 1), all of which were Bap^- and Amp^r. Two *thr*-linked mutants were Lac^+ and four were Lac^- on lactose indicator (TYE-XG) agar. All were Lac^- on lactose-MacConkey agar, which is consistent with a low level of transcription for the *phoM* operon (1, 23).

Three-factor P1 crosses showed that both Lac^+ *phoM* operon *lacZ*(Mu d1-8) fusions mapped between the *trpR* and *thr* genes. To do this, strain JC7623(R101C) [*zjj*::*cat phoM*(wt) *thr-1*] was used as a P1 donor, in which a *cat* gene had been inserted about 300 base pairs to the right of the *trpR* gene (R. Somerville, personal communication) (Fig. 1). All chloramphenicol-resistant (Cm^r) transductants that simultaneously became Thr^- were Bap^+, which is expected for a Mu d1-8 insertion that maps between the *trpR* and *thr* genes. Also, all Bap^- transductants were Thr^+, and the Lac^+ and Bap^- characters were inseparable (data not shown).

RESULTS

BAP clonal variation phenotype. An unstable Bap phenotype was noticed about 10 years ago during the construction of new *phoR* mutants by P1 transduction. When *phoR* mutations were crossed into strains W3110 and MC4100, the transductants displayed a metastable Bap phenotype. Colonies that were constitutive frequently segregated Bap^- colonies on indicator agar (TYE-XP). This instability, however, was only seen in certain strain backgrounds (Wanner, unpublished data). It seemed likely that the instability could be due to an IS element, perhaps near a *pho* gene. IS elements reside in the *proBA-lac-phoA-proC-phoBR* region; they were also thought, at the time, to contribute to the frequent formation of deletions in this region (15). Since it seemed unlikely that the variable Bap phenotype was related to its normal molecular control by phosphate limitation, strains that showed the metastable phenotype were avoided in our subsequent studies of the phosphate regulon (23).

E. coli K-12 *phoR* mutants of the XPh line show a BAP-constitutive (stable) phenotype. BAP synthesis requires the *phoM* operon in such *phoR* mutants, but not in $phoR^+$ cells (27). The *phoR* gene is part of the *phoBR* operon (14, 25) that maps near 9 min. The *phoM* operon maps near 0 min between the *trpR* and *thr* genes (24). The wild-type *phoM* operon was cloned by complementing the Bap^- Δ*phoM phoR* mutant BW2091 of the XPh lineage. However, when strain BW2091 was transformed with the multicopy *phoM* plasmids pRPG1 and pBW20, the transformants displayed an unstable Bap phenotype. It was of interest that the chromosomal DNAs that were used to make the plasmids pRPG1 and pBW20 were isolated from strains MC4100 and W3110, respectively (31). The same strains previously had given rise to *phoR* transductants that showed the unstable Bap phenotype.

Subsequent studies revealed that the apparent instability among some *phoR* transductants and in the *phoM*(wt) plasmid transformants was related. Both showed a metastable phenotype; Bap^+ colonies segregated Bap^- colonies that could give rise again to Bap^+ variants. The bidirectional switching leads to heritable changes in gene expression. The reason some *phoR* mutants showed a variable behavior and others did not was due to a genetic difference, the *pho-510* mutation, which maps in the *phoM* operon (31; this paper).

Control of BAP variability and synthesis in Cya^+ and Δ*cya* mutants with various allelic forms of the *phoM* operon. Glucose has a dramatic effect on BAP regulation in *phoR* mutants. Glucose induces BAP synthesis in variable *phoR* mutants containing the wild-type *phoM* operon, but glucose has no effect in *phoR* mutants with the *pho-510* mutant form of the *phoM* operon, which are constitutive (21; unpublished data). The effect of glucose suggested a role for catabolite repression in the control of *phoA* transcription in *phoR* mutants. Since the *pho-510* mutation led to constitutivity, that is, it mimicked the effect of added glucose, we suspected that the *phoM* operon could be involved in the glucose induction of BAP synthesis.

We tested a Δ*cya* mutation for its effect on BAP synthesis by constructing several otherwise isogenic Δ*cya* and Cya^+ *phoR* mutants with various allelic forms of the *phoM* operon. To test for variability, the mutants were plated onto a neutral BAP indicator agar (TYE-XP) (20, 21). (A neutral medium is a noninducing medium on which either clonal variant type can be propagated [20].) Several colonies of each clonal type were then quantitatively assayed for BAP activity, as described previously (20) (Table 2). Strain BW8881 [*phoR phoM*(wt)] is variable, but its nearly isogenic Δ*cya* derivative BW9083 [Δ*cya phoR phoM*(wt)] is nonvariable. To show that the loss of variability was due to the Δ*cya* mutation, Cya^+ derivatives of strain BW9083 were isolated as Mal^+ transductants after BW9083 was infected with P1 grown on a wild-type strain. Fifty Mal^+ transductants were tested, and all showed a variable Bap phenotype. We conclude that a Δ*cya* mutation abolishes BAP clonal variation.

The Bap^+ variants of strain BW8881 [*phoR phoM*(wt)] made about 10-fold more enzyme (29.5 U) than its Bap^- variants (3.0 U; Table 2). The amount (29.5 U) of enzyme in the Bap^+ variants was similar to the amount (23.1 U) in the nonvariable constitutive mutant BW8882 (*phoR pho-510*) (Table 2). However, the Bap^- variants actually made an intermediate level amount of enzyme (3.0 U), which was substantially (about 15-fold) greater than the amount in the nonvariable Bap^- mutant BW8661 [0.2 U; *phoR phoM*(Am)]

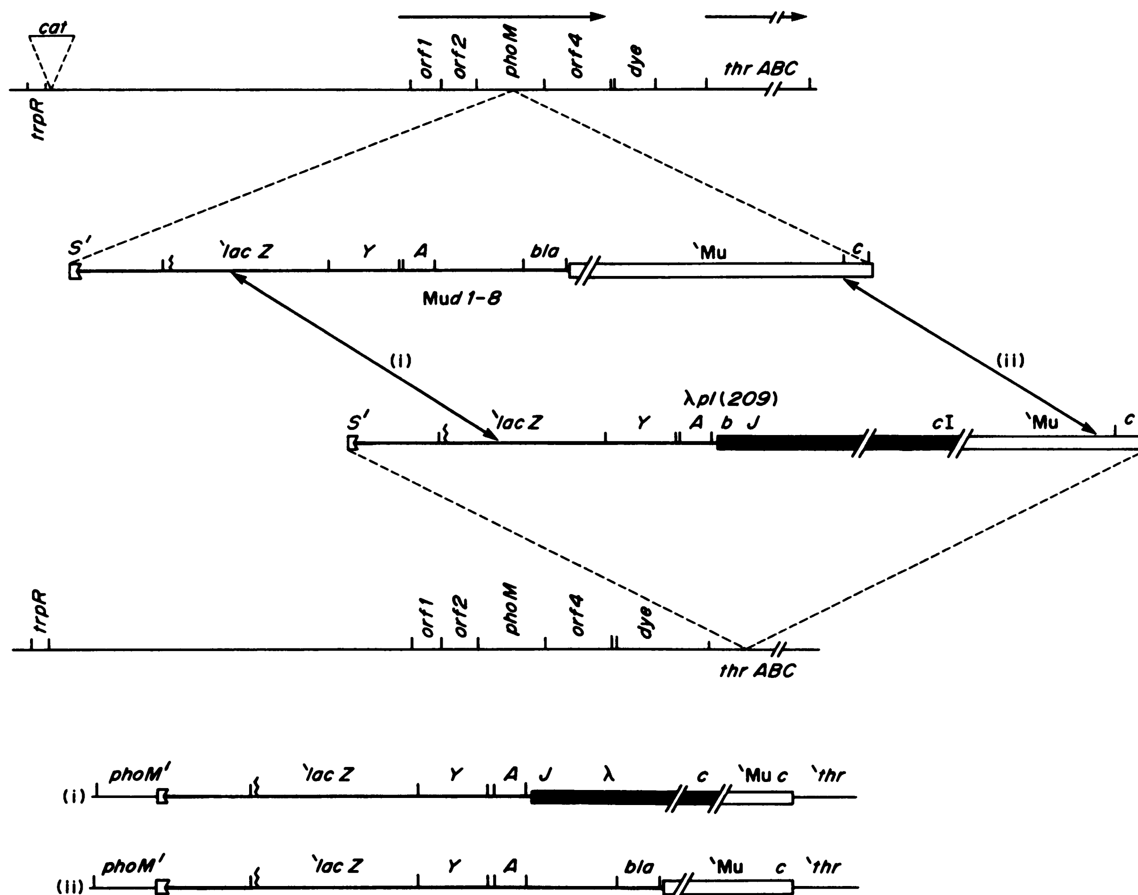

FIG. 1. Determining the orientation for *phoM-lacZY*(Mu d1-8) fusions by recombination. The top line shows the chromosomal map order in the *trpR-phoM-dye-thr* region and the site of the *zjj-101*::*cat* insertion, which was used as the selectable marker (see text). The precise locations of the Lac$^+$ Mu d1-8 insertions in the *phoM* operon were not determined. They could map in the *phoM* gene or upstream of it. In the latter case, loss of *phoM* gene function could be due to polarity. (Nonpolar mutations in ORF1 and ORF2 or insertions in ORF4 do not lead to a Bap$^-$ phenotype [unpublished data].) The structure of the Mu d1-8 phage (10) is shown in the second line, and below it the analogous structure of λp1(*209*) is illustrated. In P1 crosses in which the *cat* marker is selected, recombination could occur between homologous chromosomal sequences. Alternatively, one recombination event could occur between regions of homology that are shared by the Mu d1-8 and λp1(*209*) insertion elements. A crossover in the *lacZY* region is labeled (i); a crossover in the Mu *c* end is labeled (ii). Type (i) and (ii) recombination events could only occur if the phages were in the same relative orientation. Such crossovers would generate either type (i) deletions of the form Δ(*phoM dye thr*)::*lacZ*[λp1(*209*)] or type (ii) deletions of the form Δ(*phoM dye thr*)::*lacZ* (Mu d1-8), which are illustrated in the bottom two lines.

(Table 2). This significant amount of enzyme (3.0 U) in Bap$^-$ variants of a variable strain could be in part accountable by a mixed cell population in the variant colonies. However, this was apparently ruled out by plating the colonies onto BAP indicator agar. Although some heterogeneity was seen, the number of Bap$^+$ variant cells was generally less than a few percent of the cell population, which is severalfold lower than what would be necessary to account for the 15-fold elevation in the enzyme activity (data not shown). Therefore a different explanation is considered below.

As described above, a Δ*cya* mutation simultaneously abolishes variation and leads to a low level of BAP synthesis. The low amount (2.2 U) made in a Δ*cya phoR* mutant still requires the *phoM* operon because a *phoM*(Am) mutant made an even lower amount (0.2 U; Table 2). The amount in a Δ*cya phoR* mutant was also intermediate between the amounts in BAP-constitutive (*phoR pho-510*) and Bap$^-$ [*phoR phoM*(Am)] mutants (Table 2). These data suggest two levels of control over BAP synthesis in *phoR* mutants. One control leads to the greater amount of BAP that is made

TABLE 2. Effect of Δ*cya* on BAP synthesis and variation in *phoR* mutants

Genotype[a]	Phenotype[b]	BAP sp act[c] (U/OD_{420})	
		Mean	Range
phoM(wt)	Bap$^-$ variant	3.0	2.2–3.7
phoM(wt)	Bap$^+$ variant	29.5	26.5–31.6
Δ*cya phoM*(wt)	Bap$^+$ nonvariable	2.2	1.7–2.5
pho-510	Bap$^+$ nonvariable	23.1	18.7–27.0
Δ*cya pho-510*	Bap$^+$ nonvariable	7.5	6.6–8.8
phoM451(Am)	Bap$^-$ nonvariable	0.2	0.2
Δ*cya phoM451*	Bap$^-$ nonvariable	0.2	0.2
Δ*cya phoM453*	Bap$^-$ nonvariable	0.1	0.1

[a] All mutants have the *phoR68* allele and were derived from the *E. coli* K-12 XPh line. Strains BW8881, BW9083, BW8882, BW9082, BW8661, BW9085, and BW9084 (Table 1) were used.

[b] Bap$^-$ and Bap$^+$ variants are described in the text. Nonvariable means that all colonies were of the same phenotype.

[c] Bacteria were grown on BAP indicator agar (TYE-XP). Cya$^+$ colonies were assayed after 24 h, and Cya$^-$ colonies were assayed after 48 h as described previously (21). Four or more colonies of each type were assayed. Units are nanomoles of *p*-nitrophenol made per minute at 37°C and were determined as described previously (21). OD_{420}, Optical density at 420 nm.

TABLE 3. Effect of Δcya and Δcrp on BAP synthesis and variation in $\Delta phoM$ *phoR* mutants with various *phoM* plasmids

Genotype[a]	Plasmid[b]	Phenotype[c]	BAP sp act[d] (U/OD_{420})	
			Mean	Range
$\Delta phoM$	None	Bap^-	0.2	0.2–0.3
Δcya $\Delta phoM$	None	Bap^-	0.3	0.2–0.5
Δcrp $\Delta phoM$	None	Bap^-	0.2	0.1–0.3
$\Delta phoM$	pBW20(Var^+)	Bap^- variant	1.7	0.8–3.3
$\Delta phoM$	pBW20(Var^+)	Bap^+ variant	109.3	92.0–135.5
Δcya $\Delta phoM$	pBW20(Var^+)	Nonvariable	0.1	0.1–0.2
Δcya $\Delta phoM$	pMW1(Var^+)	Nonvariable	0.3	0.2–0.4
Δcya $\Delta phoM$	pMW1(Var^-)$_1$	Nonvariable	9.7	8.1–12.0
Δcya $\Delta phoM$	pMW1(Var^-)$_2$	Nonvariable	3.4	2.3–3.9
Δcrp $\Delta phoM$	pMW1(Var^+)	Nonvariable	0.3	0.2–0.8
Δcrp $\Delta phoM$	pMW1(Var^-)$_2$	Nonvariable	3.8	3.4–4.2
Δcya $\Delta phoM$	pRPG1(Var^+)	Nonvariable	1.0	0.8–1.5
Δcrp $\Delta phoM$	pRPG1(Var^+)	Nonvariable	1.2	0.9–1.5
Δcya $\Delta phoM$	pJP72(Var^-)	Nonvariable	10.9	10.2–12.0
Δcya $\Delta phoM$	pJP86(Var^-)	Nonvariable	21.7	19.2–24.3

[a] Strains BW2091 (DE93(*phoM*) *phoR68*), BW8877 (Δcya DE93(*phoM*) *phoR68*), and BW8898 (Δcrp DE93(*phoM*) *phoR68*) were used. The strains are derived from XPh1a (Table 1).

[b] Plasmids pBW20, pMW1, pRPG1, and pJP72 are multicopy plasmids that carry the *phoM* operon. Plasmid pJP86 carries an intact *phoM* gene, but it has a truncated form of the operon. A Var^+ plasmid carries the wild-type *phoM* region that leads to a BAP clonal variation phenotype, and a Var^- recombinant plasmid carries the *pho-510 phoM* region that leads to a constitutive (nonvariable) phenotype in *phoR* mutants (31).

[c] See footnote *b* of Table 2.

[d] See footnote *c* of Table 2.

in Bap^+ variants; this control requires that the cells be Cya^+ and $PhoM^+$. Another control acts in Δcya mutants and leads to an intermediate level of synthesis; this control also requires the *phoM* operon, however. We argue below that the wild-type *phoM* operon has both a repressor and activator role in BAP synthesis in *phoR* mutants. Such a dual control could account for the two levels of control that are described in this section.

The *pho-510* mutation abolishes both variation and the effect that glucose has on BAP synthesis in *phoR* mutants (21; unpublished data). Interestingly, the amount of BAP in a Δcya *phoR* mutant was increased about 3.4-fold in a *pho-510* mutant (Table 2). This suggests that the reduced synthesis brought about by a Δcya mutation is caused by an inhibition or repression due to the wild-type *phoM* operon. An even greater amount of enzyme was made in the nearly isogenic Cya^+ *pho-510 phoR* mutant (Table 2). That is, the *pho-510* mutation apparently does not fully restore the level of BAP synthesis in a Δcya *phoR* mutant. The data for the *pho-510* mutants in Table 2 are also consistent with two levels of control over BAP synthesis in a *phoR* mutant: (i) a *cya*-dependent control that is *phoM* dependent and (ii) a *cya*-independent control that is also *phoM* dependent.

Control of BAP synthesis in Δcya *phoR* and Δcrp *phoR* mutants with various *phoM* plasmids. We showed above that a Δcya mutation abolished BAP clonal variation; it also reduced the amount of BAP synthesis. Furthermore, the *phoM* operon seemed to be involved, because the *pho-510* mutant form of the *phoM* operon increased BAP synthesis in a Δcya mutant. To further define how catabolite repression and the *phoM* operon regulated BAP synthesis in *phoR* mutants, we examined the effects of various *phoM* plasmids in nearly isogenic Δcya Δ(*phoM*)*93 phoR* and Δcrp Δ(*phoM*)*93 phoR* mutants. [The Δ(*phoM*)*93* deletion removes the entire *phoM* operon along with the neighboring *trpR*, *dye*, *thr*, and *chlG* genes (24).] Two kinds of *phoM* plasmids were tested (31). One type contains the wild-type *phoM* operon that leads to a metastable Bap phenotype. These are identified as Var^+ plasmids and include pBW20, pRPG1, and pMW1(Var^+). The other type contains the *pho-510* mutant form of the *phoM* operon, which are identified as Var^- plasmids and include pJP72, pJP86, and pMW1(Var^-). Our data below also support a dual role for the *phoM* operon in the control of BAP synthesis in *phoR* mutants. A negative control was seen in cells with the wild-type *phoM* operon; a positive control was seen in cells with the *pho-510* mutant form of the *phoM* operon.

The $\Delta phoM$ *phoR* mutant BW2091 was Bap^- on a neutral BAP indicator agar (TYE-XP). As expected, its Δcya and Δcrp derivatives were also Bap^- (Table 3). The Var^+ plasmid pBW20 [*phoM*(wt)] (31) transformants of strain BW2091 were metastable; they switched between a Bap^+ and Bap^- phenotype (Table 3). When the same plasmid DNA was transformed into a nearly isogenic Δcya $\Delta phoM$ *phoR* mutant, the transformants were nonvariable and Bap^- (Table 3). A nonvariable phenotype was expected because the Δcya *phoR phoM*(wt) mutant BW9083 was nonvariable above (Table 2). However, the Bap^- phenotype of these *phoM*(wt) plasmid transformants suggested that the wild-type *phoM* operon exerted a negative control over BAP synthesis in *phoR* mutants, as described below.

The Δcya *phoR* mutant BW9083 containing the wild-type *phoM* operon on the chromosome made an amount of enzyme that was intermediate between the amounts made in the Bap^- mutant BW8661 and the Bap^+ mutant BW8882, which are nonvariable (Table 2). However, Δcya $\Delta phoM$ *phoR* transformants containing a multicopy *phoM*(wt) plasmid were Bap^- (Table 3). If the wild-type *phoM* operon encoded a repressor, then the Bap^- phenotype could be due to the high copy number of the wild-type *phoM* operon when it was carried on a plasmid. To test this possibility, we transformed strain BW9083 (Δcya *phoR*) with a multicopy *phoM*(wt) plasmid. These transformants made about sixfold less enzyme than strain BW9083 itself (data not shown), showing that multiple copies of the wild-type *phoM* operon further reduced BAP synthesis. In other words, a Δcya mutation abolished BAP variation in *phoR* mutants that contain the wild-type *phoM* operon, regardless of whether the *phoM*(wt) operon was on the chromosome or a multicopy plasmid. But BAP synthesis was reduced in a Δcya

mutant when the *phoM*(wt) operon was present in multicopy. These data are consistent with a negative regulatory role for the wild-type *phoM* operon in controlling BAP synthesis in *phoR* mutants. Apparently, a greater level of repression was seen in Δ*cya phoR* mutants carrying a multicopy *phoM*(wt) plasmid (Table 3).

There was also a 15-fold increase in BAP synthesis in a Δ*cya phoR* mutant containing the wild-type *phoM* operon over the amount made in *phoM phoR* mutants (Table 2). This shows that the *phoM* operon has an activator role. However, a chromosomal copy of the *pho-510* mutant form of the *phoM* operon increased BAP synthesis (by 3.4-fold) above the amount in a Δ*cya phoR phoM*(wt) mutant. The further increase due to the *pho-510* mutation is consistent with the *phoM* operon having both a positive and negative regulatory role (Table 2). That is, the greater synthesis in Δ*cya phoR pho-510* mutants could be due to loss of negative control by the *pho-510* mutant form of the *phoM* operon. We therefore examined the effect of the *pho-510 phoM* operon when it was on a multicopy plasmid. To do this, we compared the nearly identical pMW1(Var$^+$) and two pMW1(Var$^-$) *phoM* plasmids. [These Var$^-$ plasmids were made by recombining the *pho-510* mutation(s) onto pMW1(Var$^+$) by homologous recombination (31).] The pMW1(Var$^-$) plasmids increased by 32- and 11-fold the amount of BAP made in a Δ*cya* Δ*phoM phoR* mutant (Table 3). Since an increase was only seen in Δ*cya* Δ*phoM phoR* mutants that were transformed with the *pho-510 phoM* plasmids, the reduction in BAP synthesis seen in a Δ*cya phoR* mutant apparently involves a negative control by the wild-type *phoM* operon. This conclusion was reached because the *pho-510 phoM* operon shows epistasis to the Δ*cya* mutation. We conclude that the wild-type *phoM* operon both negatively and positively regulates BAP synthesis in *phoR* mutants.

To determine whether Crp was also involved in the control of BAP synthesis in a *phoR* mutant, we examined the effect of a Δ*crp* mutation. Like a Δ*cya* mutation, a Δ*crp* mutation abolished variation and led to a Bap$^-$ phenotype in a Δ*phoM phoR* mutant containing the *phoM*(wt) plasmid pMW1(Var$^+$) (Table 3). The nearly identical pMW1(Var$^-$) plasmid containing the *pho-510 phoM* operon led to a 13-fold increase in the amount of BAP made (Table 3). We conclude that the effects of a Δ*cya* and Δ*crp* mutation on BAP synthesis in a *phoR* mutant are similar.

We examined several *phoM* plasmids to be sure that the effects were not specific to the plasmid replicon. The pBW20 and pMW1 plasmids are pBR322 derivatives and behaved similarly (Table 3). The plasmids pRPG1, pJP72, and pJP86 were made with the pACYC184 vector and contain different amounts of the chromosomal *phoM* region. pRPG1 has the wild-type *phoM* operon on it and leads to a variable Bap phenotype; pJP72 and pJP86 were made with DNA from the *pho-510* mutant form of the *phoM* operon (31). pRPG1 [*phoM*(wt)] transformants of a Δ*cya* Δ*phoM phoR* and Δ*crp* Δ*phoM phoR* mutant were nonvariable and made a small amount of BAP (Table 3). A severalfold greater amount of BAP was made in Δ*cya* Δ*phoM phoR* mutants containing the *pho-510 phoM* plasmids pJP72 and pJP86 (Table 3). We conclude that a Δ*cya* and Δ*crp* mutation abolish BAP variation; either simultaneously reduces BAP synthesis in *phoR* mutants containing the wild-type *phoM* operon. Also, the decrease in BAP synthesis brought about in a *phoR* mutant by a Δ*cya* or Δ*crp* mutation involves the *phoM* operon. This is because the *pho-510* mutant form of the *phoM* operon is epistatic; the *pho-510* mutation allows for an increase in BAP synthesis in Δ*cya phoR* and Δ*crp phoR* mutants. An effect of the *pho-510* mutation was especially dramatic when the *phoM* operon is on a multicopy plasmid. Apparently, this is caused by a greater level of repression due to the *phoM*(wt) plasmid in Δ*cya phoR* and Δ*crp phoR* mutants.

Attempts to show that cAMP directly regulates BAP synthesis in *phoR* mutants gave ambiguous results, however. Strains BW8881 [*phoR phoM*(wt)] and BW9083 [Δ*cya phoR phoM*(wt)] were grown on BAP indicator agar (TYE-XP) with various concentrations of cAMP (0.1 to 2.0 mM was used at about twofold increments, in medium that was adjusted to pH 7.5 to increase the uptake of cAMP [26]). The Cya$^+$ strain BW8881 [*phoR phoM*(wt)] showed a variable Bap phenotype, regardless of the extracellular cAMP concentration. Also, occasional Bap$^+$ (blue) variants were seen with the Δ*cya phoR phoM*(wt) mutant BW9083 in the presence of cAMP, and none was seen in its absence. However, it was not possible to purify even a few of the presumed Bap$^+$ variants, either in the presence or absence of cAMP. Instead, all apparent Bap$^+$ colonies tested gave streaks that essentially contained only Bap$^-$ variants. Our lack of unequivocal data could mean that maintaining the Bap$^+$ state requires cellular regulation of the cAMP levels. However, an indirect effect of cAMP and Crp on BAP synthesis could also account for our results. That cAMP was entering the cells was evident because the Δ*cya* mutant grew more rapidly in its presence.

Characterizing and determining the orientation of *phoM* operon *lacZ*(Mu d1-8) fusions. Since the *phoM* operon is required for BAP synthesis in *phoR* mutants (27), we considered that both the variation and the effects of a Δ*cya* and Δ*crp* mutation could be due to changes in *phoM* operon expression. We used two *phoM* operon *lacZ* transcriptional fusions to test this possibility. Both fusions were identified as Bap$^-$ Lac$^+$ Mu d1-8 insertions that mapped between the *trpR* and *thr* genes, as described in Materials and Methods. Since the *phoM* operon is transcribed clockwise (23), we showed that they were fused to the *phoM* operon promoter by verifying that the Mu d1-8 insertions had a clockwise orientation. Their expression of the *lacZ* reporter gene therefore should accurately reflect the transcription of the *phoM* operon. The experiments that were done to determine the orientation also showed that the *pho-510* mutation maps upstream of the *phoM* (ORF3) gene.

We demonstrated that the *phoM* operon *lacZ*(Mu d1-8) insertions had the same clockwise orientation as a Lac$^+$ Mu-*lacZ* insertion in the *thr* operon that is nearby (1, 2, 24). This was done by showing that deletions could be formed by recombination between Lac$^+$ Mu-*lacZ* elements in the *phoM* and *thr* operons. Since the *phoM-thr* region is nonessential (24), recombination between homologous *lac* or Mu sequences of various Mu-*lac* elements inserted in this region could generate deletions. However, this should occur only if the Mu-*lac* insertions are in the same relative orientation (Fig. 1). To test for the formation of deletions, we did P1 crosses between strains with *phoM* operon *lacZ* fusions and a strain with a *thrA*::*lacZ* fusion. We used as a selectable marker a nontransposable *cat* gene, containing its own promoter, which was inserted near the *trpR* gene (R. Somerville, personal communication). Donor strains had a Lac$^+$ *phoM* operon *lacZ*(Mu d1-8) insertion, and the recipient had a Lac$^+$ *thr*::*lacZ* [λp1(*209*)] mutation. The *thr*::*lacZ*(λp1(*209*) insertion was made by replacing the *thr*::*lacZ*(Mu d1) mutation of strain BW2246 (31) with the Mu-*lac* λp1(*209*) phage, as described previously (28). We had previously shown that the same *thr*::*lacZ*(Mu d1) insertion contained the Mu d1 transposon in the clockwise orientation by molecular cloning

TABLE 4. Determining the orientation for Lac$^+$ *phoM-lacZ* fusions via P1 transduction

Transductant class[a]	No. of transductants[b] with P1 grown on:			Phenotype[c]
	phoM-lacZ$_1$	*phoM-lacZ*$_2$		
		Cross 1	Cross 2	
A	19	10	34	Bap$^+$(Var$^+$) Amp^s Dye^r Thr$^-$ λ^{imm}
B	14	11	18	Bap$^-$ Amp^r Dye^r Thr$^+$ λ^s
C	13	5	4	Bap$^-$ Amp^s Dye^s Thr$^-$ λ^{imm}
D	0	1	2	Bap$^-$ Amp^r Dye^s Thr$^-$ λ^s
E	6	2	4	Bap$^-$ Amp^r Dye^r Thr$^-$ λ^{imm}
F	0	1	7	Bap$^+$(Var$^-$) Amp^s Dye^r Thr$^-$ λ^{imm}
Total	52	30	69	

[a] P1 was grown on *phoM*::*lacZY*(Mu d1-8)$_1$ or *phoM*::*lacZY*(Mu d1-8)$_2$ fusion strains and used to infect the strain BW10189 containing a *thr*::*lacZ*[λp1(*209*)] insertion. CM^r transductants that acquired the chromosomal *zjj-101*::*cat* insertion (Fig. 1; unpublished data) were purified once and then tested for various markers in the *trpR-zjj-phoM-dye-thr* region (see text). The transductants corresponded to six recombinant types: class A showed the recipient phenotype, which are recombined between the *zij*::*cat* insertion and the *phoM* operon; class B showed the donor phenotype, which are recombined on the clockwise side of the *thr* operon; class C showed the phenotype of a deletion-recombinant type (i) in Fig. 1, which have the Δ(*phoM-dye-thr*)::*lacZ*[λp1(*209*)] deletion that was generated via recombination within the Mu *c* ends of the Mu d1-8 and λp1(*209*) phages; class D showed the phenotype of the deletion-recombinant type (ii) in Fig. 1, which have the Δ(*phoM-dye-thr*)::*lacZ*(Mu d1-8) deletion that was generated via recombination within the *lacZ* end of the Mu d1-8 and λp1(*209*) phages; class E corresponded to recombination events that occurred within the chromosomal sequences between the *phoM* and *thr* insertion sites, thereby creating recombinants containing both the *phoM*::*lacZY* (Mu d1-8) and *thr*::*lacZY*[λp1(*209*)] fusions; and class F showed the nonvariable (Var$^-$), BAP-constitutive phenotype that is characteristic of *phoR* mutants with the *pho-510* allele. The class F transductants, presumably, arose by recombination between the *pho-510* and *phoM*::*lacZ*(Mu d1-8) mutations in the donor DNA, as described in the text.

[b] Strain BW10128 was the donor of the *phoM*::*lacZ*(Mu d1-8)$_1$ fusion; strain BW9836 was the donor in cross 1 and strain BW10129 was the donor in cross 2 for the *phoM*::*lacZ*(Mu d1-8)$_2$ fusion.

[c] Bap$^+$(Var$^+$), Bap$^-$, and Bap$^+$(Var$^-$) indicate BAP-variable, -negative, and constitutive (nonvariable) phenotypes, respectively. Amp^r and Amp^s phenotypes are due to the presence or absence of the *bla* gene between the *lacZY* and Mu *c* end of the Mu d1-8 phage (Fig. 1). The Dye^r and Dye^s phoenotypes are due to the *dye* gene, the loss of which leads to growth sensitivity on toluidine blue agar (6). λ^{imm} or λ^s indicates that the recombinant is immune or sensitive to λcIh*80*, respectively. All recombinants were tested and shown to be ChlG$^+$, which requires a gene to the right of the *thr* operon.

of its upstream DNA which included the *phoM* operon (22, 31).

Phage P1 was grown on strain BW10128 [*zjj*::*cat phoM*::*lacZ*(Mu d1-8)$_1$] as well as strains BW9836 and BW10129, both of which have *zjj*::*cat* near the *phoM*::*lacZ*(Mu d1-8)$_2$ fusion. Strain BW10189 {*phoM*(wt) *thr*::*lacZ*[λp1(*209*)]*phoR*} was infected with these lysates, and Cm^r transductants were selected; the transductants were tested for various markers in the *phoM-thr* region. (Although we selected for chloramphenicol resistance, the crosses could have been done by selecting for the ampicillin resistance marker of Mu d1-8.) If the Mu-*lac* elements are in the same relative orientation, then crossovers between Mu d1-8 and λp1(*209*) sequences would generate *phoM* operon *lacZ*Δ(*phoM-thr*)::[λp1(*209*)Ts$^+$] or *phoM* operon *lacZ*Δ(*phoM-thr*)::(Mu d1-8) fusion-deletion-type recombinants, which are identified as deletion type (i) or (ii) recombinants, respectively, in Fig. 1. Such deletions should be Dye^s and show the respective BAP, Amp, λ, and Thr phenotypes. Over 60% of the transductants had marker phenotypes for the *phoM-thr* region which corresponded to the parental types, i.e., the class A and B transductants in Table 4. Both *phoM-lacZ1* and *phoM-lacZ2* fusions also yielded several Dye^s transductants that had phenotypes corresponding to type (i) or (ii) recombinants (Fig. 1). These are identified as class C or D transductants, respectively, in Table 4. This is significant because such transductants could arise by homologous recombination only if the *phoM* operon and *thr-lacZ* fusions were in the same orientation.

The class E recombinants probably arose by recombination of chromosomal sequences between the sites of the Mu-*lac* insertions in the *phoM* and *thr* operons. This transductant class would therefore contain both Mu-*lac* elements in tandem on the chromosome, an arrangement that would make this class unstable. As a consequence, the class E transductants could undergo a secondary recombination event that would segregate type (i) or (ii) deletions. The substantial numbers of deletion type recombinants (i.e., class C and D transductants) as primary transduction events made it unnecessary to test the class E transductants for their genetic stability. However, this would provide an alternate way to verify the relative orientation of two Mu-*lac* elements.

The frequent occurrence of the class C and D transductants (Table 4) shows that the Lac$^+$ Mu-*lac* insertions in the *phoM* and *thr* operons are in the same relative orientation. Additional experiments were done to prove that these transductant classes had true deletions, by showing that they behaved as deletions when used as donors in P1 crosses. To do this, P1 was grown on one class C recombinant and one class D recombinant that resulted from each cross. Strain BW3627 [*phoM*(wt) *phoR* Δ*lac*] was infected, and Cm^r transductants were selected. In each case, the Bap$^-$ Lac$^+$ Dye^s Thr$^-$ markers were simultaneously transferred along with the expected ampicillin resistance or λ immune markers. These data show that the class C and class D transductants correspond to the type (i) and (ii) deletions in Fig. 1, respectively. Since the class C and D deletion recombinants were ChlG$^+$, the *chlG* gene maps outside the *phoM-thr* interval. Its position with respect to the *phoM-thr* region had been uncertain (24).

Determining the *pho-510* map location. The class F transductants in Table 4 show the BAP-constitutive phenotype that is characteristic of a *phoR* mutant with the *pho-510* mutation in the *phoM* operon (21). The *thr*::*lacZ* strain that was used as a recipient contained the wild-type *phoM* operon. However, the *phoM* operon *lacZ* fusions were made in a *phoR* mutant with the *pho-510* mutant form of the *phoM* operon. (A *phoR pho-510* mutant was used to allow detecting *phoM* operon insertions as Bap$^-$ mutants, as described in the Materials and Methods.) Therefore we reasoned that a crossover between the *pho-510* mutation(s) and the Mu-*lacZ* insertion in the donor could have occurred, thus leading to constitutive transductants. A single crossover event could generate the class F recombinants if the *pho-510* mutation mapped within the interval between the *zjj*::*cat* and *phoM*

TABLE 5. Expression of *phoM-lacZ* transcriptional fusions in BAP-variable and -constitutive *phoR* mutants

Genotype[a]	Plasmid	Phenotype[b]	BAP sp act[c] (U/OD_{420})	β-Galactosidase sp act[d] (U/OD_{420})
phoM-lacZ$_1$	None	Bap^-	ND[e]	4.8
Δ*cya phoM-lacZ*$_1$	None	Bap^-	ND	2.8
phoM-lacZ$_2$	None	Bap^-	ND	3.6
Δ*cya phoM-lacZ*$_2$	None	Bap^-	ND	3.1
phoM-lacZ$_1$	pMW1(Var^+)	Bap^- variant	0.9	4.3
phoM-lacZ$_1$	pMW1(Var^+)	Bap^+ variant	32.0	3.5
Δ*cya phoM-lacZ*$_1$	pMW1(Var^+)	Bap^-	0.3	4.3
phoM-lacZ$_2$	pMW1(Var^+)	Bap^- variant	1.0	4.3
phoM-lacZ$_2$	pMW1(Var^+)	Bap^+ variant	32.4	5.0
Δ*cya phoM-lacZ*$_2$	pMW1(Var^+)	Bap^-	0.3	5.7
phoM-lacZ$_1$	pMW1(Var^-)$_2$	Bap^+(Var^-)	63.4	3.2
Δ*cya phoM-lacZ*$_1$	pMW1(Var^-)$_2$	Bap^+(Var^-)	99.2	4.8
phoM-lacZ$_2$	pMW1(Var^-)$_2$	Bap^+(Var^-)	85.8	7.0
Δ*cya phoM-lacZ*$_2$	pMW1(Var^-)$_2$	Bap^+(Var^-)	62.8	5.0

[a] All bacteria were derived from the *E. coli* K-12 BD792 unmutagenized line (see text) and were also *phoR68* and *recA1*. The strains BW9593, BW9594, BW9595, and BW9596 were used, either lacking a plasmid or with the indicated plasmid.

[b] See footnote *b* of Table 2 and footnote *c* of Table 4. Cells were grown on BAP indicator (TYE-XP) agar with kanamycin, as appropriate.

[c] See footnote *c* of Table 2.

[d] Units for β-galactosidase activity are nanomoles of *o*-nitrophenol made per minute at 28°C and were determined as described previously (29). At least four colonies of each type were assayed; the mean values are given.

[e] ND, Not done. The same mutants made about 0.5 U of BAP per unit of optical density at 420 nm in other experiments.

operon *lacZ*(Mu d1-8) insertion. Since the Mu d1-8 insertion leads to a Bap^- phenotype, the Mu d1-8 insertion maps either within the *phoM* (ORF3) gene or upstream of it, in which case the *phoM* gene function could be abolished due to polarity.

We previously showed that the *pho-510* mutation maps within the *phoM* operon or 500 base pairs preceding the ORF1 translation start site (31). Also, the *pho-510* mutation is recessive (31). Therefore it probably leads to a loss of function and the *pho-510* mutation could not map in the *phoM* (ORF3) gene itself. The finding of class F recombinants in crosses with the *phoM* operon *lacZ*(Mu d1-8)$_2$ insertion are therefore consistent with a map location for the *pho-510* mutation in either ORF1 or ORF2. The lack of the class F transductants in the *phoM-lacZ*$_1$ cross could imply that this fusion maps further upstream. However, it is also possible that strain BW10128, which was used as a donor of the *phoM-lacZ*$_1$ fusion, may not carry the *pho-510* mutation because its construction involved several crosses of the *phoM* region between bacteria that contain the wild-type *phoM* operon (data not shown).

BAP clonal variation and expression of the *phoM* operon. Since a *phoM* mutation abolishes BAP synthesis in *phoR* mutants, the amount of BAP made could reflect the level of *phoM* operon expression. We used the two *phoM* operon *lacZ* fusions that were described above to determine whether the level of *phoM* operon expression was associated either with the effects of a Δ*cya* mutation or the alternating Bap phenotype. We examined the relationship between BAP synthesis and *phoM* operon expression by transforming Cya^+ *phoR* and Δ*cya phoR* mutants, which contained a *phoM* operon *lacZ* fusion, with nearly identical *phoM*(wt) and *pho-510 phoM* plasmids. Both *phoM-lacZ* fusions were examined, since they could map in different genes within the *phoM* operon. All mutants lacking a plasmid were Bap^- and expressed the *phoM* operon *lacZ* fusions at a low level (Table 5). When the *phoM*(wt) pMW1(Var^+) plasmid was transformed into a Δ*cya phoM-lacZ phoR* mutant, the transformants remained Bap^-. The same plasmid also failed to restore BAP synthesis in a Δ*cya* Δ*phoM phoR* mutant, as described above. There was also no significant effect on the expression of the *phoM* operon *lacZ* fusion (Table 5). Similar data were obtained with a Δ*crp phoR* mutant containing a *phoM* operon *lacZ* fusion (data not shown). We conclude that Δ*cya* and Δ*crp* mutations are unlikely to affect *phoM* operon expression, at least not in Bap^- *phoR* mutants as determined with these *phoM* operon *lacZ* fusions.

The *phoR* mutants containing either *phoM* operon *lacZ* fusion showed the variable Bap phenotype when transformed with the *phoM*(wt) pMW1(Var^+) plasmid. This allowed testing for an association between the Bap phenotype and the level of *phoM* operon expression. No change in *phoM* operon expression accompanied the alternating Bap phenotype (Table 5). There was also no evidence of an independent variation for the Lac phenotype on Lac indicator agar (TYE-XG; data not shown). We conclude that BAP variation is not associated with an alternation in *phoM* operon expression, at least not when the cells are complemented by a multicopy *phoM*(wt) plasmid and *phoM* operon expression is monitored in *trans*.

We showed above that the *phoM* mutation largely overcame the effect that a Δ*cya* or Δ*crp* mutation had on reducing BAP synthesis in *phoR* mutants. Therefore we examined the effect of a *pho-510 phoM* plasmid on *phoM* operon expression in Cya^+ and Δ*cya* mutants that contained a *phoM* operon *lacZ* fusion. Both *phoM* operon *lacZ* fusions were tested. The transformants made with the pMW1(Var^-) plasmid were fully constitutive; and there was no effect of a Δ*cya* mutation (Table 5). There was also no significant effect on *phoM* operon expression in *trans*. It should be pointed out that the experiments in Table 5 were done with nearly isogenic mutants of the *E. coli* K-12 BD792 lineage, whereas the data in Table 3 were obtained with strains in the XPh1a lineage. This could be the cause of some quantitative differences. Nevertheless, the results in these tables are qualitatively similar. We conclude that the *pho-510* mutation fully restores BAP synthesis in Δ*cya phoR* mutants, at least in the more nearly wild-type BD792 lineage. At the same time, *phoM* operon expression is not affected in *trans*.

DISCUSSION

BAP synthesis is normally regulated by the *phoBR* and *pstSCAB-phoU* operons in response to phosphate limitation

(23). In *phoR* mutants, however, *phoA* gene expression no longer responds to phosphate. Instead, the BAP clonal variation phenotype is seen (20, 21, 31). The *phoR* mutant cells alternately show a Bap$^+$ and Bap$^-$ phenotype when grown on a neutral (noninducing) medium. This control acts on transcription of the BAP structural gene, *phoA* (unpublished data). Also, the *phoR* mutant variability is epistatic to mutations that map in *pstSCAB-phoU* and *phoF* regions (19–21; unpublished data). The variation is somehow controlled by the *phoM* operon (21, 31) whose DNA sequence shows four ORFs in an operon structure (1). The *phoR* and *phoM* (ORF3) gene products are thought to act similarly in the activation of the *phoA* gene (23, 27). The similarity at the protein sequence level (16) provides additional support for a common molecular basis for transcriptional activation by PhoR and PhoM.

One interesting feature of the BAP clonal variation phenotype of *phoR* mutants is the physiological regulation of variation. The growth medium, especially the presence of glucose, dramatically affects the pattern of variation. When *phoR* mutants that are otherwise wild type are grown on a glucose-minimal agar, they are generally Bap$^+$ and do not show variation. That is, BAP synthesis in *phoR* mutants is induced by glucose. This induced synthesis leads to constitutivity that is heritable when the cells are replated in the absence of glucose (20, 21; unpublished data), thus showing that the induction leads to a constitutive state in the cells (21). On certain other media, *phoR* mutants may express a Bap$^-$ phenotype, which is also heritable when the cells are replated on a neutral medium (unpublished data). The molecular basis for the variation and the apparent induction of the alternative Bap$^+$ and Bap$^-$ states is not understood.

The effect of glucose on BAP synthesis and variation (21) suggested that catabolite repression could regulate *phoA* gene expression in *phoR* mutants. In this paper, we examined the effects of catabolite repression in two ways. (i) We made Δ*cya phoR* and Δ*crp phoR* mutants of the XPh1a lineage with various chromosomal alleles of the *phoM* operon. (ii) We transformed Δ*cya phoR* and Δ*crp phoR* mutants, of both the XPh1a and BD792 lineages, with multicopy Var$^+$ *phoM*(wt) and Var$^-$ *pho-510 phoM* plasmids. Since the BD792 mutants also contained a *phoM* operon *lacZ* transcriptional fusion on the chromosome, the latter set of experiments allowed us to monitor simultaneously *phoM* operon expression in *trans*.

To examine *phoM* operon expression, it was important to show that the *phoM* operon *lacZ* transcriptional fusions accurately reflected transcription from the *phoM* operon promoter. However, we could not show this by examining their regulation because there is no known control over *phoM* operon expression. Instead we used a genetic approach. We showed that both Lac$^+$ Mu d1-8 insertions mapped within the *phoM* operon because they led to a Bap$^-$ phenotype in *phoR* mutants and mapped between the *trpR* and *thr* genes. To show that the fusions were expressed from the *phoM* operon promoter, we verified their orientation on the chromosome. This involved transductional crosses between strains containing Mu-*lac* elements in the *phoM* and *thr* operons that are nearby. We reasoned that if the Mu-*lac* transposons were in the same relative orientation, then a proportion of the transductants would have recombined within the Mu or *lac* sequences that are in common. Such an event would lead to a deletion (Fig. 1). Three crosses gave a substantial proportion of transductants showing the expected phenotypes of the two deletion types that were anticipated; the resultant mutations also behaved as deletions in subsequent crosses. We concluded that both *phoM* operon *lacZ* fusions are in the same clockwise orientation as the *thr* operon, as expected for a fusion to the *phoM* operon (23).

The method we used for determining the orientation of Mu-*lac* insertions may be generally useful. Three other methods were previously described (11, 13, 30). The ones described by MacNeil (13) and Hughes and Roth (11) also depended upon homologous recombination between Mu or Mu-*lac* elements that were nearby. MacNeil's procedure required the use of several Mu insertions in the vicinity of the Mu-*lac* insertion (14). Hughes and Roth (11) determined the orientation of Mu-*lac* insertions by constructing chromosomal duplications in *Salmonella typhimurium*. However, the ability to make the necessary duplications (with a reasonable amount of effort) may depend upon the use of phage P22 (11), which, unlike the *E. coli* phage P1, is unable to encapsidate an entire Mu-*lac* element. The scheme that Hughes and Roth (11) described for making site-directed deletions is equivalent to the one that we used to determine the orientation of the *phoM* operon *lacZ* fusions.

In this paper we showed that a Δ*cya* and Δ*crp* mutation abolished BAP clonal variation in *phoR* mutants, regardless of whether the wild-type *phoM* operon was on the chromosome or a multicopy plasmid. An effect of cAMP on variation could not be directly demonstrated, however. Equivocal results were found when cAMP was added to a Δ*cya* mutant. Nevertheless, it is reasonable to propose that cAMP and its receptor protein do control variation because deletion of either the adenyl cyclase or the *crp* gene abolishes variation. The alternative possibility that other genes near both the *cya* and *crp* loci are responsible for the variation seems unlikely, but it was not excluded.

The amount of BAP made in Δ*cya phoR* and Δ*crp phoR* mutants depended upon whether the wild-type *phoM* operon was present in single or multiple copies. A Δ*cya phoR* and Δ*crp phoR* mutant with a single copy of the wild-type *phoM* operon on the chromosome made a reduced amount of enzyme. This amount was further reduced about 10-fold in nearly isogenic mutants with a multicopy *phoM*(wt) plasmid. Indeed, Δ*cya* Δ*phoM phoR* and Δ*crp* Δ*phoM phoR* transformants carrying a multicopy *phoM*(wt) plasmid were Bap$^-$. Also, the presence of a multicopy *phoM*(wt) plasmid decreased BAP synthesis in Δ*cya phoR* cells having a chromosomal copy of the wild-type *phoM* operon. These data are consistent with a negative role for the wild-type *phoM* operon in the control of BAP synthesis in *phoR* mutants. In addition, our results provided further support for a positive regulatory role for the *phoM* operon in gene expression.

Two kinds of experiments were done to determine the mechanism by which catabolite repression controls BAP synthesis in *phoR* mutants. The first involved testing the effect of a Δ*cya* mutation in *phoR* mutants with various allelic forms of the *phoM* operon. We showed that the *pho-510* mutant form of the *phoM* operon largely restored the level of BAP synthesis in a Δ*cya phoR* mutant; it increased the amount made 3.4-fold over that made by a nearly isogenic mutant with the wild-type *phoM* operon on the chromosome (Table 2). However, a much greater effect of the *pho-510* mutation was seen in Δ*cya phoR* and Δ*crp phoR* mutants that contained multicopy *phoM*(wt) and *pho-510 phoM* plasmids. With the *phoM* plasmids, the *pho-510* mutation increased the amount made from 11-fold to over 100-fold, depending upon which strain and plasmid were tested (Tables 3 and 5). These data are consistent with an activator role for the *pho-510 phoM* operon. Since the

pho-510 phoM operon mutation is recessive, it is apparently defective in repression. We conclude that the wild-type *phoM* operon has a dual regulatory role, in repression and activation of BAP synthesis in *phoR* mutants. Accordingly, the *pho-510* mutation may define a gene involved in repression. The epistatic nature of the *pho-510* mutation in Δ*cya phoR* and Δ*crp phoR* mutants implies that adenyl cyclase and Crp may indirectly control BAP synthesis via an interaction with the *phoM* operon or its gene products.

We considered that the amount of BAP made could reflect differences in the level of *phoM* operon expression. If so, BAP clonal variation could reflect an alternation in *phoM* operon expression. This hypothesis was also tested with two *phoM* operon *lacZ* transcriptional fusions. No significant effect on *phoM* operon expression was seen when Cya^+ *phoR* and Δ*cya phoR* mutants, carrying a chromosomal *phoM* operon *lacZ* fusion, were transformed with nearly identical *phoM*(wt) and *pho-510 phoM* plasmids. (i) There was no change in *phoM* operon transcription that paralleled the alternation in BAP synthesis. (ii) A Δ*cya* mutation did not alter *phoM* expression, even though the Δ*cya* mutation abolished variation. (iii) There was also no effect on *phoM* operon expression in the presence of the wild-type *phoM* or *pho-510 phoM* operon in *trans*. We concluded that the effects on BAP synthesis in *phoR* mutants are probably not accountable by a change in *phoM* operon expression. There was also no evidence for autogenous control by the wild-type *phoM* operon.

The variation of BAP synthesis in *phoR* mutants could be explained if proteins encoded in the *phoM* operon and the cAMP-Crp complex controlled another locus which in turn caused variation. Alternatively, the *phoM* operon product(s) may directly control variation in which case the cAMP-Crp system could, either directly or indirectly, alter a *phoM* operon protein(s). In this regard, it is interesting that both PhoM (ORF3) and adenyl cyclase are probably associated with the membrane (1, 7). Such a location could allow for a direct interaction between these proteins.

Whatever the actual molecular mechanism is, Δ*cya* and Δ*crp* mutations affect an alternating pattern of gene expression in bacteria. Perhaps other variable phenomena are similarly regulated by the cAMP-Crp protein complex. If the *phoM* operon is involved, its effect may only be seen in bacteria with the wild-type *phoM* operon (21, 31). However, such an effect of the *phoM* operon could have previously gone unnoticed. This is because the *pho-510* mutation, like the *spoT1* and *relA1* mutations (12), is present in numerous common laboratory strains of *E. coli* K-12, including many that were used in studies of cAMP, Crp, and catabolite repression (21). A variety of strain-dependent effects of catabolite repression (26) could be due to the widespread occurrence of the *pho-510* mutation. Whether other cAMP and Crp-dependent phenomena are affected by the *pho-510* mutation or are subject to variation is unknown.

Based upon the effects of glucose (21), adenyl cyclase, Crp, and *phoM* operon mutations on BAP variation and synthesis in *phoR* mutants, we propose that the *phoM* operon may encode regulatory proteins that respond to catabolite repression. Accordingly, protein products of the *phoM* operon may normally regulate a class of catabolite responsive promoters in addition to affecting the phosphate regulon in *phoR* mutants. The results in this paper indicate that the *phoM* operon has a regulatory role in both gene activation and repression. The *pho-510* mutation may abolish BAP clonal variation by eliminating the catabolite control over BAP synthesis, which is seen only in *phoR* mutants.

ACKNOWLEDGMENTS

We thank B. Bachmann, J. Beckwith, J. Roth, and H. E. Umbarger for generously providing bacterial strains, especially R. Somerville for providing strain R101C before publication, and J. Sheridan for secretarial assistance. We thank I. Tessman for carefully reading an earlier version of the manuscript.

M.R.W. is a David Ross Graduate Fellow at Purdue University. This work was supported by Public Health Service award GM35392 from the National Institutes of Health.

LITERATURE CITED

1. **Amemura, M., K. Makino, H. Shinagawa, and A. Nakata.** 1986. Nucleotide sequence of the *phoM* region of *Escherichia coli*: four open reading frames may constitute an operon. J. Bacteriol. **168**:294–302.
2. **Bachmann, B. J.** 1987. Linkage map of *Escherichia coli* K-12, edition 7, p. 807–877. *In* F. C. Neidhardt, J. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium* cellular and molecular biology. American Society for Microbiobiology, Washington, D.C.
3. **Bachmann, B. J.** 1987. Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12, p. 1190–1219. *In* F. C. Neidhardt, J. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
4. **Brickman, E., and J. Beckwith.** 1975. Analysis of the regulation of *Escherichia coli* alkaline phosphatase synthesis using deletions and φ80 transducing phages. J. Mol. Biol. **96**:307–316.
5. **Brickman, E., L. Soll, and J. Beckwith.** 1973. Genetic characterization of mutations which affect catabolite-sensitive operons in *Escherichia coli*, including deletions of the gene for adenyl cyclase. J. Bacteriol. **116**:582–587.
6. **Buxton, R. S., and L. S. Drury.** 1983. Cloning and insertional inactivation of the *dye*(*srfA*) gene, mutation of which affects sex factor F expression and dye sensitivity of *Escherichia coli* K-12. J. Bacteriol. **154**:1309–1314.
7. **de Crombrugghe, B., S. Busby, and H. Buc.** 1984. Cyclic AMP receptor protein: role in transcription activation. Science **224**: 831–838.
8. **Drury, L. S., and R. S. Buxton.** 1985. DNA sequence analysis of the *dye* gene of *Escherichia coli* reveals amino acid homology between the Dye and OmpR proteins. J. Biol. Chem. **260**:4236–4242.
9. **Fassler, J. S., I. Tessman, and E. S. Tessman.** 1985. Lethality of the double mutations *rho rep* and *rho ssb* in *Escherichia coli*. J. Bacteriol. **161**:609–614.
10. **Hughes, K. T., and J. R. Roth.** 1984. Conditionally transposition-defective derivative of Mu*d*1(Amp Lac). J. Bacteriol. **159**:130–137.
11. **Hughes, K. T., and J. R. Roth.** 1985. Directed formation of deletions and duplications using Mu*d*(Ap, *lac*). Genetics **109**: 263–282.
12. **Laffler, T., and J. Gallant.** 1974. *spoT*, a new genetic locus involved in the stringent response in *E. coli*. Cell **1**:27–30.
13. **MacNeil, D.** 1981. General method, using Mu-Mud1 dilysogens, to determine the direction of transcription of and generate deletions in the *glnA* region of *Escherichia coli*. J. Bacteriol. **146**:260–268.
14. **Makino, K., H. Shinagawa, and A. Nakata.** 1985. Regulation of the phosphate regulon of *Escherichia coli* K-12: regulation and role of the regulatory gene *phoR*. J. Mol. Biol. **184**:231–240.
15. **Riley, M., and A. Anilionis.** 1978. Evolution of the bacterial genome. Annu. Rev. Microbiol. **32**:519–560.
16. **Ronson, C. W., B. T. Nixon, and F. M. Ausubel.** 1987. Conserved domains in bacterial regulatory proteins that respond to environmental stimuli. Cell **49**:579–581.
17. **Simon, M. I., J. Zieg, M. Silverman, G. Mandel, and R. Doolittle.** 1980. Phase variation: evolution of a controlling element. Science **209**:1370–1374.
18. **Wanner, B. L.** 1983. Overlapping and separate controls on the

phosphate regulon in *Escherichia coli* K12. J. Mol. Biol. **166**:283–308.

19. **Wanner, B. L.** 1986. Novel regulatory mutants of the phosphate regulon in *Escherichia coli* K-12. J. Mol. Biol. **191**:39–58.
20. **Wanner, B. L.** 1986. Bacterial alkaline phosphatase clonal variation in some *Escherichia coli* K-12 *phoR* mutant strains. J. Bacteriol. **168**:1366–1371.
21. **Wanner, B. L.** 1987. Control of *phoR*-dependent bacterial alkaline phosphatase clonal variation by the *phoM* region. J. Bacteriol. **169**:900–903.
22. **Wanner, B. L.** 1987. Molecular cloning of Mu*d*(*bla lacZ*) transcriptional and translational fusions. J. Bacteriol. **169**:2026–2030.
23. **Wanner, B. L.** 1987. Phosphate regulation of gene expression in *Escherichia coli*, p. 1326–1333. *In* F. C. Neidhardt, J. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
24. **Wanner, B. L., and J. Bernstein.** 1982. Determining the *phoM* map location in *Escherichia coli* K-12 by using a nearby transposon Tn*10* insertion. J. Bacteriol. **150**:429–432.
25. **Wanner, B. L., and B.-D. Chang.** 1987. The *phoBR* operon in *Escherichia coli* K-12. J. Bacteriol. **169**:5569–5574.
26. **Wanner, B. L., R. Kodaira, and F. C. Neidhardt.** 1978. Regulation of *lac* operon expression: reappraisal of the theory of catabolite repression. J. Bacteriol. **136**:947–954.
27. **Wanner, B. L., and P. Latterell.** 1980. Mutants affected in alkaline phosphatase expression: evidence for multiple positive regulators of the phosphate regulon in *Escherichia coli*. Genetics **96**:353–366.
28. **Wanner, B. L., and R. McSharry.** 1982. Phosphate-controlled gene expression in *Escherichia coli* K12 using Mu*d1*-directed *lacZ* fusions. J. Mol. Biol. **158**:347–363.
29. **Wanner, B. L., A. Sarthy, and J. Beckwith.** 1979. *Escherichia coli* pleiotropic mutant that reduces amounts of several periplasmic and outer membrane proteins. J. Bacteriol. **140**:229–239.
30. **Wanner, B. L., S. Wieder, and R. McSharry.** 1981. Use of bacteriophage transposon Mu*d1* to determine the orientation for three *proC*-linked phosphate-starvation-inducible (*psi*) genes in *Escherichia coli* K-12. J. Bacteriol. **146**:93–101.
31. **Wanner, B. L., M. R. Wilmes, and E. Hunter.** 1988. Molecular cloning of the wild-type *phoM* operon in *Escherichia coli* K-12. J. Bacteriol. **170**:279–288.