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Bacterial Alkaline Phosphatase Clonal Variation in Some *Escherichia coli* K-12 *phoR* Mutant Strains†

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Several *phoR* alleles (*phoR19*, *phoR20*, *phoR68*, *phoR69*, *phoR70*, and *phoR78*) led to either a bacterial alkaline phosphatase (BAP)-constitutive phenotype or a variable behavior, depending upon the strain tested. Whereas *Escherichia coli* K10, MC1000, and XPh4 *phoR* mutants were constitutive, AB1157, BD792, MC4100, and W3110 *phoR* mutants displayed the metastable character. For the latter strains, constitutive mutants regularly segregated BAP-negative clones which yielded constitutive variants again at a high frequency. Indeed, the pattern of variation observed in BAP-variable *phoR* strains is phenotypically analogous to phase variation of the H1/H2 flagellum antigen type in *Salmonella typhimurium* and the molecular switch between the immune and sensitive states in bacteriophage lambda. The metastable behavior was not a general property of BAP-constitutive mutants, since several phosphate-specific transport-*phoU* mutations led to a constitutive (stable) phenotype regardless of the strain tested. But in *phoR* phosphate-specific transport-*phoU* mutants, the metastable character was epistatic (dominant), and such double mutants showed clonal variation in BAP-variable strains.

Bacterial alkaline phosphatase (BAP) is a classic example of an inducible protein made in bacteria. Its synthesis is induced more than 1,000-fold when cells are stressed by phosphate limitation; when the cells are starved, as much as 6% of the cell protein made is BAP. This induction allows growth of *Escherichia coli* on a wide variety of organic phosphate esters. BAP-constitutive mutants were isolated over 25 years ago, and Hfr crosses defined two loci, R1 (*phoB-phoR*) at 9 min and R2 (phosphate-specific transport [PST]-*phoU*) at 84 min, which are involved in its control (5). R1 mutants are of three types. (i) R1a mutants, such as *phoR68*, are constitutive but only make about one-third the fully induced amount of enzyme, even when starved; (ii) R1b mutants, such as *phoR69*, are fully constitutive; and (iii) R1c mutants, which map in *phoB* (a nearby gene), are uninducible. However, all R2 mutants behave like classic repressor mutants and either make the maximally induced amount of enzyme when starved or are already fully constitutive (10, 19, 21). Importantly, R1a (*phoR*) and R1c (*phoB*) alleles are epistatic in R2 mutants; i.e., the appropriate double mutants make the lower amount of enzyme characteristic of the R1 allele (4). Most R1 mutations were of the R1a type, including several amber alleles (5). Later, R2 mutants were shown to be defective in the PST system (26); it was also reported that they probably regulate BAP synthesis indirectly, perhaps via the synthesis of an effector molecule. Since BAP synthesis is also induced by guanine starvation in a manner dependent upon the *phoR* allele, a guanine compound may act as an effector molecule in regulating BAP expression (25).

In several studies of BAP expression, various isogenic *E. coli* XPh4 mutants were used (17–24). (In earlier reports, strains are often designated as XPh1a derivatives, although they are more closely related to XPh4, which in turn is a descendant of strain XPh1a [2].) In other laboratories, *E. coli* K10 strains were frequently used. Both are descendants of the standard *E. coli* K-12 strain (1, 2), and the corresponding *phoR* mutants synthesize BAP constitutively. However, when the same *phoR* mutations, including nonsense alleles, were transferred into other *E. coli* K-12 strains, it was often not possible to find truly constitutive recombinants. Instead, *phoR* transductants displayed a novel BAP phenotype. These mutants showed clonal variation of BAP expression in which variants alternated between constitutive and negative states with respect to BAP synthesis.

BAP clonal variation in *phoR* mutants is described here along with several strains showing either the variable or the constitutive (stable) phenotype. Conditions for testing this behavior are also described. Whatever the precise molecular mechanism for BAP regulation is, it must also account for this phenomenon.

MATERIALS AND METHODS

Media. BAP variation was studied for cells grown at 37°C on TYE medium containing 10 g of tryptone (Difco Laboratories), 5 g of yeast extract (Difco), 8 g of NaCl, and 15 g of Bacto-Agar (Difco) per liter. TYEG medium also contained 1% glucose. XP (5-bromo-4-chloro-3-indolyl-phosphate-*p*-toluidine; Bachem) was added by spreading 75 μl of a 20 mg/ml solution (in dimethyl formamide) onto the surface. (For other media used, see reference 17.)

Bacterial strains. The genotypes and derivations of new strains are listed in Table 1. The pedigree gives the designation of the parental strain (obtained from another laboratory) as well as the immediate ancestor of the strain at this laboratory. For other strains used, see references cited or Bachmann (1). All stock cultures were routinely stored at −70°C in Luria-Bertani broth with 8% dimethyl sulfoxide.

Test for BAP variation. In general, variability was tested by colony purification on TYE-XP agar (Fig. 1). Once variants were noticed, each type was purified twice or more consecutively to yield nearly homogeneous streaks, and the opposite variant type was chosen and similarly purified to homogeneity; the original type was then selected again. This

† This paper is dedicated to the memory of the late Hiroshi Inouye, Temple University, who died 24 July 1986.

TABLE 1. Bacterial strains

Strain	Genotype[a]	Pedigree (reference)[b]	Source or construction[c]
BW9, XPh301	*phoR68 rpsL thi crp-72*	XPh4	23
BW255	*phoR68 rpsL thi crp-72*	XPh4	
BW257	*phoR70*(Am) IN(*rrnD-rrnE*)*1* Δ*lac-169 malT rpsL*	W3110 via BW136 (17)	Pro$^+$ with P1 grown on C6
BW258	IN(*rrnD-rrnE*)*1* Δ*lac-169 malT rpsL phoR70*(Am)	W3110 via BW136	Pro$^+$ with P1 grown on C6
BW261	*phoR19* IN(*rrnD-rrnE*)*1* Δ*lac-169 malT rpsL*	W3110 via BW136	Pro$^+$ with P1 grown on C29
BW269	*phoR68* IN(*rrnD-rrnE*)*1* Δ*lac-169 rpsL malT*	W3110 via BW136	Pro$^+$ with P1 grown on R21W (BW111 [21])
BW304	*phoR68 rpsL thi phoM453 crp-72*	XPh4 via R16 (BW89 [21])	Lac$^+$ with P1 grown on W3110
BW337	*phoR68* IN(*rrnD-rrnE*)*1* Δ*lac-169 malT*	W3110 via BW317 (17)	Pro$^+$ with P1 grown on BW255
BW361	IN(*rrnD-rrnE*)*1 ara* Δ*lac-169 proC*::Tn*5 tsx trpA9605 his-29 malT ilv-1 trpR*	FE103 via BW350 (22)	Aro$^+$ with P1 grown on W3110
BW391	IN(*rrnD-rrnE*)*1 ara* Δ*lac-169 tsx trpA9605 his-29 malT ilv-1 trpR*	FE103 via BW361	Pro$^+$ with P1 grown on W3110
BW453	IN(*rrnD-rrnE*)*1 ilv-1 his-29*(Am) *trpA9605 trpR tsx ara malT* Δ*lac-169 proCYA221*	FE103 via BW391	Pro$^-$ with P1 grown on CA7087 and penicillin enrichment
BW478	IN(*rrnD-rrnE*)*1* Δ*lac-169 phoR68 tpo-11*	W3110	20
BW503	*phoR68 rpsL thi phoM451*(Am) *crp-72*	XPh4	Lac$^+$ with P1 grown on BW304
BW507	IN(*rrnD-rrnE*)*1 ara* Δ*lac-169 phoR70*(Am) *tsx trpA9605 his-29 malT ilv-1 trpR*	FE103 via BW361	Pro$^+$ with P1 grown on C6
BW508	IN(*rrnD-rrnE*)*1 ara* Δ*lac-169 phoR19*(Am) *tsx trpA9605 his-29 malT ilv-1 trpR*	FE103 via BW361	Pro$^+$ with P1 grown on C29
BW511	IN(*rrnD-rrnE*)*1 ara* Δ*lac-169 phoR20*(Am) *tsx trpA9605 his-29 malT ilv-1 trpR*	FE103 via BW361	Pro$^+$ with P1 grown on C36
BW531	IN(*rrnD-rrnE*)*1* Δ*lac-169 phoR69 tsx trpA9606 trpR his-29 ara malT ilv-1*	FE103	20
BW646	Δ*lac-169 proC*::Tn*5 rpsL crp-72 aroB ilv-691*::Tn*10 thi*	XPh4 via BW576 (24)	Tc^r with P1 grown on DB6960
BW1023	IN(*rrnD-rrnE*)*1* Δ*lac-169 phoR68 trpA9605 trpR his-29 ilv-1 malT ara*(?)	FE103 via BW453	Pro$^+$ with P1 grown on BW478
BW1508	*phoR68* IN(*rrnD-rrnE*)*1* Δ*lac-169 trpA9605 trpR his-29 malT ara*(?)	FE103 via BW1023	Ilv$^+$ with P1 grown on BW503
BW3212	Δ*lac-169* Δ(*psiF proC phoB phoR*)*9-6 thi*	XPh4 via BW1965 (24)	Aro$^+$ with P1 grown on W3110
BW3370	Δ*lac-169 proC*::Tn*5*	BD792	Kan^r with P1 grown on BW574 (20)
BW3376	Δ*lac-169 proC*::Tn*5*	BD792	Kan^r with P1 grown on BW574
BW3378	*phoR68 trpR thrB proC*::Tn*5* Δ*lac-169*	W3110 via M4126	Kan^r with P1 grown on BW574
BW3414	Δ*lac-169*	BD792 via BW3376	Pro$^+$ spontaneously
BW3627	Δ*lac-169 phoR68*	BD792	17
BW3732	Δ*lac-169 proR69*	BD792 via BW3370	Pro$^+$ with P1 grown on BW531
BW3850	Δ*lac-169 ilv-691*::Tn*10*	BD792 via BW3414	Tc^r with P1 grown on BW646
BW3869	*phoR69* Δ*lac-169 his*::Tn*10 hsrR17 endA1 supE44 thi*	MM294 via DB6989	Pro$^+$ with P1 grown on BW531 (20)
BW3891	*phoS21* Δ*lac-169*	BD792 via BW3850	Ilv$^+$ with P1 grown on CGSC5009
BW3893	*phoS27* Δ*lac-169*	BD792 via BW3850	Ilv$^+$ with P1 grown on CGSC5650
BW3895	*phoS28* Δ*lac-169*	BD792 via BW3850	Ilv$^+$ with P1 grown on CGSC5651
BW3944	*phoR78* Δ*lac-169*	BD792 via BW3370	Pro$^+$ with P1 grown on CGSC6439
BW4047	*phoR69* Δ*lac-169 hsdR17 endA1 supE44 thi*	MM294 via BW3869	His$^+$ spontaneously
BW5809	*phoR70*(Am) Δ*lac-169 thi*	XPh4 via BW1902 (19)	Pro$^+$ with P1 grown on BW258
BW5841	Δ*lac-169 phoR69 thi*	XPh4 via BW3212	Pro$^+$ with P1 grown on BW531
BW5922	*phoR20*(Am) Δ*lac-169*	BD792 via BW3370	Pro$^+$ with P1 grown on C36
BW5923	*phoR19*(Am) Δ*lac-169*	BD792 via BW3370	Pro$^+$ with P1 grown on C29
BW6098	*phoR68 rpsL thi crp-72 tsx*::Tn*10*	XPh4 via BW255 (21)	Tc^r with P1 grown on P2595 (17)
BW6126	*phoR68 tsx-234*::Tn*10* Δ*lac-169 rpsL150 relA1 araD139 thi? flbB5301 deoC1 ptsF25*	MC4100	Tc^r with P1 grown on BW6098
BW6415	*phoR68 ilv-691*::Tn*10* Δ*lac-169*	BD792 via BW3627	Tc^r with P1 grown on BW646
BW6509	*phoR68 phoS21* Δ*lac-169*	BD792 via BW6415	Ilv$^+$ with P1 grown on CGSC5009
BW6512	*phoS27 phoR68* Δ*lac-169*	BD792 via BW6415	Ilv$^+$ with P1 grown on CGSC5650
BW6514	*phoS28 phoR68* Δ*lac-169*	BD792 via BW6415	Ilv$^+$ with P1 grown on CGSC5651
BW6516	*pho-30*(Am) *phoR68* Δ*lac-169*	BD792 via BW6415	Ilv$^+$ with P1 grown on CGSC5653
BW7756	*phoR68 tsx*::Tn*10 thr-1 leu-6 thi-1 supE44 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE3 rpsL31 sup-37*	AB1157	Tc^r with P1 grown on BW6098
C3F2	*phoR69*(?) *rpsL*		A. Garen via J. Gallant
DB6989	*his*::Tn*10 hsdR17 endA1 supE44 pro-82 thi*	MM294	D. Botstein
GR5230	*rpsL phoR68*		M. Malamy
M4126	IN(*rrnD-rrnE*)*1 thrB trpR*	W3110	J. Gardner
SM138	*phoR68* Δ*lac-169 rpsL150 relA1 araD139 thi? flbB5301 deoC1 ptsF25*	MC4100	S. Michaelis
SM527	*phoR68 araD139* Δ(*ara-leu*)*7679* Δ(*lac-3*)*X74 galU galK rpsL thi proC*::Tn*5*	MC1000	S. Michaelis
XPhla	*proR68 lacZ524 rpsL thi crp-72 trpC*(Oc)		J. Beckwith

[a] IN, Inversion; Oc, ochre mutation.

[b] The pedigree gives the designation of the parental strain (obtained from another laboratory) as well as the immediate ancestor of the strain at this laboratory.

[c] Strains C6, C29, C36, CGSC5009, CGSC5650, CGSC5651, CGSC6439, and CGSC5653 were obtained from A. Garen via B. Bachmann, who also assigned the *pho* allele numbers. Tc^r and Kan^r indicate tetracycline or kanamycin resistance, respectively.

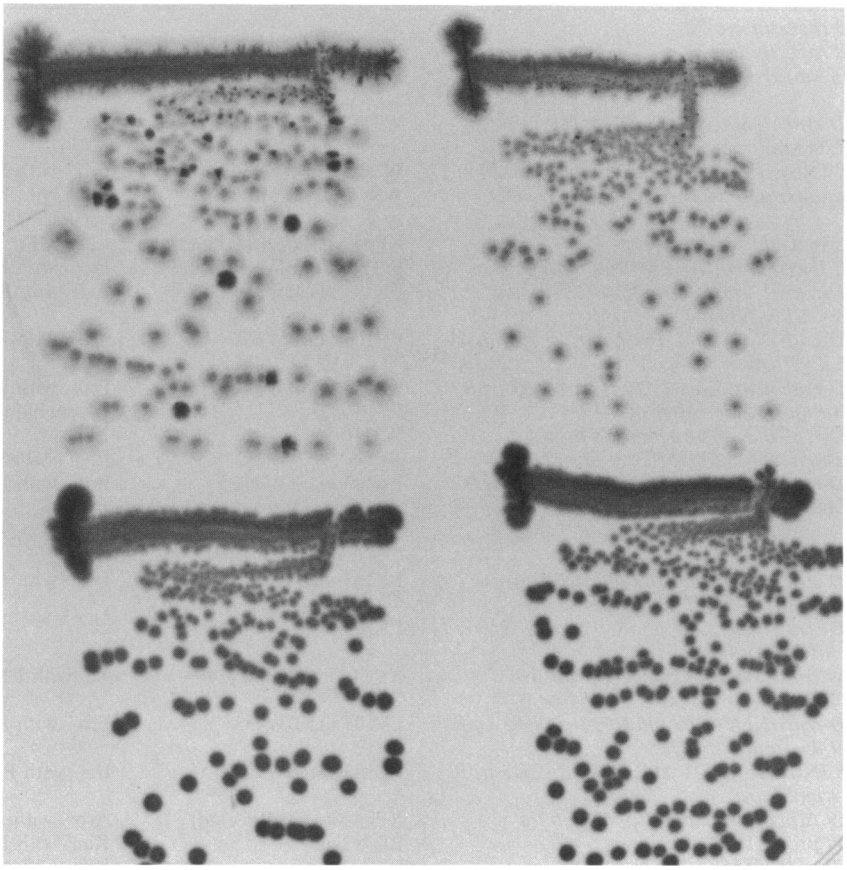

FIG. 1. Clonal variation of BAP expression. BAP-negative (top) and -constitutive (bottom) clonal variants of strain BW3627 were streaked on TYE-XP agar. The darker colonies made about 50-fold more enzyme than the lighter colonies (see text).

scheme constituted one complete cycle. In the cases studied here, three or more complete cycles of BAP variation were demonstrated. Usually, several variants of each strain were tested simultaneously. This was important because, in a few cases, the testing itself yielded apparent second-site mutants which no longer allowed the cells to show variation (unpublished results). The basis of this is uncertain. It should also be noted that PST-*phoU* BAP-constitutive mutants occasionally segregated clones making lesser amounts of enzyme. However, these variants were stable and, in several cases, were shown to have secondary mutations in either *phoB*, *phoR*, or *phoA* (unpublished results). (Stable *phoR* mutants were only noticed in PST-*phoU* strains of a nonvariable line, as expected.)

Cell growth and enzyme assays. The amounts of BAP activity were measured for individual colonies grown at 37°C for 16 to 24 h on TYE-XP agar. To do this, colonies were suspended either in ~0.3 ml of 0.85% saline or in assay buffer to give an optical density at 420 of about 0.2 to 0.4. Portions were removed to measure the turbidity (with a microcuvette in a Gilford 260 spectrophotometer [Gilford Instrument Laboratories, Inc.]), BAP activity, and sometimes viable cell counts. (Saline suspensions were used for determining the number of viable cells.) Cells were lysed with chloroform-sodium dodecyl sulfate, and BAP activity was measured in 1 M Tris hydrochloride (pH 8.0) with *p*-nitrophenylphosphate as the substrate. Assay tubes were incubated until an A_{410} of 0.2 to 0.5 was reached or for up to 24 h, after which BAP was inhibited by adding 0.67 M KH_2PO_4 (final), and the tubes were chilled in an ice bath. Cell debris was removed by centrifugation, and the absorbance was measured as described previously (17). Units are nanomoles of *p*-nitrophenol made per minute at 37°C.

Genetics. Bacteriophage P1-mediated transductions were done by using P1 *kc* as previously described (17). At least 10 transductants were tested in each cross.

RESULTS

Clonal variation of BAP synthesis in a *phoR* strain. Strain BW3627 had an R1a mutation (*phoR68*) and showed clonal variation of BAP synthesis. Constitutive clones segregated BAP-negative variants which again gave rise to constitutive variants at a high frequency. Whereas this strain produced almost completely homogeneous colony populations in either state, negative variants of *phoR* BD792 mutants tended to yield constitutive variants more frequently (Fig. 1 and data not shown). However, some other strains tended to yield negative variants more frequently (data not shown). The frequency of variation for strain BW3627 varied from undetectable ($<10^{-5}$) to over 10%, depending upon the colony age and growth medium (data not shown). The amounts of BAP made in the constitutive and negative variants differed more than 50-fold when determined in small colonies less than 12 h old and when expressed as the amount of enzyme activity per viable cell (data not shown).

Variability among various *E. coli* K-12 laboratory strains. It was of interest why this striking variation in BAP expression was previously unnoticed in *phoR* mutants. To investigate this, various *phoR* laboratory stocks were tested for the

TABLE 2. Phenotype of various *phoR* mutant strains

Strain[a]	BAP phenotype	Allele	Prototype[b]
AB1157	Variable	*phoR68*	BW7756
BD792	Variable	*phoR19*(Am)	BW5923
	Variable	*phoR20*(Am)	BW5922
	Variable	*phoR68*	BW3627
	Variable	*phoR69*	BW3732
	Variable	*phoR78*	BW3944
C3F2	Constitutive	*phoR69*[c]	
FE103[d]	Variable	*phoR19*(Am)	BW508
	Variable	*phoR20*(Am)	BW511
	Variable	*phoR68*	BW1508
	Constitutive	*phoR69*	BW531
	Variable	*phoR70*(Am)	BW507
GR5230	Constitutive	*phoR68*	
M4126	Variable	*phoR68*	BW3378
MC1000	Constitutive	*phoR68*	SM527
MC4100	Variable	*phoR68*	SM138, BW6126
MM294[e]	Constitutive	*phoR69*	BW4047
W3110	Variable	*phoR19*(Am)	BW261
	Variable	*phoR68*	BW269
	Variable	*phoR68*	BW337
	Variable	*phoR70*(Am)	BW257
XPhla	Variable[f]	*phoR68*	
XPh4	Constitutive	*phoR68*	BW9, BW5810
	Constitutive	*phoR69*	BW5841
	Constitutive	*phoR70*	BW5809

[a] All the K10 R1a and R1b mutants tested were constitutive (data not shown). See footnote *c* to Table 1 for some K10 strains tested.

[b] See Table 1 for derivations.

[c] This strain supposedly contains the *phoR69* allele from strain C3; however, C3F2 did not yield *phoM*-independent transductants when crossed into a suitable *phoM* mutant (unpublished results).

[d] The variation in these strains is less dramatic on indicator media because the constitutive amount of synthesis is reduced (see text).

[e] Results with this strain are tentative since the only *phoR* allele tested shows less frequent variation in some other strains (see text).

[f] BAP-negative variants of this strain were noticed less often and were more difficult to purify.

BAP-variable character. Altogether over 100 different *phoR* strains were tested, including several closely related strains. As summarized in Table 2, BAP variation was observed in five laboratory lines, including AB1157, BD792, MC4100, W3110, and XPh1a, and in two lines derived from W3110, i.e., FE103 and M4126 (Table 1). (Strain BD792 is the parent of strain BW3627.) Under the conditions tested, i.e., growth on TYE-XP agar, variation was most noticeable in BD792 and MC4100 mutants. No variation was observed, however, for *phoR* mutants of four other lines tested. These included several K10 *phoR* mutants (data not shown; see Table 1, footnote *c* for strains tested), an F^- strain used by Garen (e.g., CSF2 [5]), an F^- strain used by Malamy (e.g, GR5230 [27]), and strain XPh4 used by this laboratory. The variation was also generally independent of the *phoR* allele tested, although *phoR69*-variable mutants tended to yield fewer variants in some cases. Also, among R1a mutations, both amber mutations (*phoR19*, *phoR20*, and *phoR70*) and nonsuppressible alleles (*phoR68* and *phoR78*) caused variation in a variable strain. Even the R1b *phoR69* allele caused variation when tested in the *phoR*-variable BD792 line.

BAP expression in constitutive and negative clonal variants. The amounts of enzyme made in constitutive and negative variants and in constitutive (stable) *phoR* mutants are shown in Table 3. Although the amounts of constitutive expression varied greatly among strains, all constitutive strains showed substantially more BAP activity. For the highly variable BD792 and MC4100 strains, the amounts of enzyme made by constitutive variants were 30-fold or more greater than the amounts made by negative variants (Table 3 and data not shown). Also, constitutive *phoR69* variants make more enzyme than isogenic and constitutive *phoR68* variants, and this was also true in constitutive (stable) *phoR* strains (21). The reduced synthesis in BAP-negative *phoR69* variants may reflect the apparent reduced frequency of variation in strain BW3732. The BW3627 variants which were induced by growth on TYEG medium made amounts of enzyme similar to the amounts made by the constitutive variants of this strain. (Since all colonies showed constitutive synthesis immediately after growth on TYEG medium, it appears that the constitutive state is inducible [data not shown].) However, constitutive *phoR* variants of FE103, a W3110 derivative, made substantially less enzyme, and this could be the reason why so many W3110 *phoR* mutants were more difficult to classify (Table 2).

Epistasis of *phoR* allele in PST-*phoU* mutants of a variable strain. A collection of well-characterized PST-*phoU* mutations were similarly tested for variation, but none showed variation in any strain examined. The dominant character was, therefore, determined by constructing several *phoR* PST-*phoU* double mutants of a variable strain. The results clearly show that the *phoR*-variable character was epistatic (Table 4). (In each case, the presence of both the PST-*phoU* and *phoR* mutations was confirmed by backcrossing.) In addition, the *phoR* mutants of a variable line showed variation in a *phoU* mutant and in a PST-*phoU*-deleted strain (data not shown). When analogous *phoR* PST-*phoU* mutants were constructed in a nonvariable line, the double mutants were constitutive, i.e., stable (data not shown).

DISCUSSION

Strain-dependent *phoR* clonal variation of BAP synthesis. All *E. coli* K10 *phoR* mutants tested showed a constitutive (stable) phenotype with respect to BAP synthesis. Also, strain XPh4 derivatives were stable. This is important since numerous studies on BAP regulation were done in these K-12 lines. In addition, MC1000 *phoR* mutants showed a constitutive (stable) phenotype. However, at least four other lines displayed a metastable character; in *E. coli* AB1157, BD792, MC4100, and W3110, *phoR* mutants switched alternately between constitutive and negative states with respect to BAP synthesis. Since variable *phoR* mutants were used in many studies of BAP expression, much of that work may now need to be reevaluated, as indicated below.

Presumably, the variable and constitutive (stable) strains differ in some unlinked gene which is responsible for their different phenotypes. One difference maps in the *phoM* locus (B. L. Wanner, submitted for publication). Interestingly, BD792 and W3110 were the most nearly wild-type strains tested here (1; B. J. Bachmann, personal communication), and both showed variation. The metastable *phoR* phenotype, therefore, appears to be associated with wild-type *E. coli* K-12. Apparently, the stable character evolved in laboratory strains. Whether there exists a common progenitor showing the constitutive (stable) *phoR* character for some strains studied here is unknown. But in the case of XPh4, the stable mutation may have occurred independently, since it was derived from the variable strain XPh1a via mutagenesis (2).

Several physiological factors dramatically affected BAP variation. For instance, the constitutive state was induced in negative variants by growth on TYEG agar; after growth on

TABLE 3. BAP synthesis in *phoR* mutants

Strain (prototype)	Allele	Type[a]	Medium	BAP activity (U at OD_{420})[b]		No. of colonies tested
				Mean	Range (values)	
AB1157 (BW7756)	*phoR68*	Pho^+	TYE	31.9	(33.4, 30.3)	2
		Pho^-	TYE	3.0	(2.49, 3.60)	2
BD792 (BW3627)	*phoR68*	Pho^+	TYE	47.6	37.6–56.1	4
		Pho^-	TYE	1.4	1.23–1.54	4
		Pho^{+c}	TYEG	118	102–136	4
		Pho^{-c}	TYEG	53.6	34.4–70.6	4
BD792 (BW3732)	*phoR69*	Pho^+	TYE	132	126–137	4
		Pho^-	TYE	0.03	0.02–0.03	4
BD792 (BW3944)	*phoR78*	Pho^+	TYE	24.9	(23.1, 26.6)	2
		Pho^-	TYE	1.5	(1.42, 1.58)	2
FE103 (BW1508)	*phoR68*	Pho^+	TYE	5.6	(4.90, 6.31)	2
		Pho^-	TYE	1.7	(1.61, 1.75)	2
FE103 (BW507)	*phoR70*	Pho^+	TYE	6.6	(5.45, 7.69)	2
		Pho^-	TYE	1.3	(1.23, 1.36)	2
MC1000 (SM527)	*phoR68*	Constitutive	TYE	50.8	48.4–54.1	4
W3110 (BW337)	*phoR68*	Pho^+	TYE	21.5	(20.4, 22.6)	2
		Pho^-	TYE	1.4	(1.25, 1.55)	2
XPh4 (BW9)	*phoR68*	Constitutive	TYE	70.7	64.9–82.6	4

[a] Pho^+ and Pho^- refer to constitutive and negative BAP variants as described in the text.
[b] Units are nanomoles of *p*-nitrophenol made per minute at 37°C in 1 M Tris (pH 8.0) buffer (21). OD_{420}, Optical density at 420 nm.
[c] Colony type prior to incubation on TYEG medium.

inducing medium constitutive variants predominated and sometimes were the only kind observed upon subsequent repurification on TYE agar. This apparent inheritable change in gene expression was due to an actual induction process, since it was not accompanied by a loss of cell viability (data not shown). The induction was probably a catabolic response, since a similar effect was observed with complex maltose medium, but only in maltose-utilizing strains (data not shown). Several other growth shifts, e.g., minimal to rich media, starvation, etc., also appeared to cause a dramatic shift to the constitutive state in some strains. Older colonies and overnight growth in LB broth often yielded mixtures, regardless of the variant tested. Also, even among variable strains, there were differences in the pattern of variation. Depending upon the strain, either the constitutive or the negative state tended to predominate, at least under the conditions tested. Nevertheless, the frequent repurification of colonies (sometimes at 10- to 12-h intervals on prewarmed media) could generally yield more or less homogeneous populations of either variant in *phoR*-variable strains (data not shown).

Implications of BAP variation on its regulation. The variation of *phoA* expression observed in *phoR* mutants is phenotypically analogous to phase variation in *Salmonella typhimurium* (18) or to the molecular switch between the immune and sensitive states in bacteriophage lambda development (13). Although these processes are phenotypically very similar, their molecular controls are quite different indeed. In H2/H1 antigen variation, a site-specific DNA inversion occurs at the H2 locus, and the orientation of the invertible element simultaneously controls expression of H2 and the unlinked H1 locus (12). However, no direct evidence exists for a DNA rearrangement which affects phosphate control. The switch is *recA* independent, however (unpublished results). The molecular switch controlling the immune and sensitive states in bacteriophage lambda involves the *cI* and *cro* regulatory proteins, which compete for overlapping operators. Whether *cI* or *cro* binds preferentially controls the expression of a bidirectional promoter region controlling their syntheses, thus maintaining either the immune or the sensitive state (11). Whether similar protein-DNA interactions control BAP expression is unknown. Such a control could involve competition between activator and repressor proteins, or transcription termination factors may be involved. Indeed, the induced change of state which occurs in *phoR* BAP-negative variants on TYEG medium is phenotypically similar to the thermal induction of the sensitive state in bacteriophage lambda mutant lysogens (9, 13).

TABLE 4. Epistasis of *phoR* variation in PST-*phoU* mutants

Strain[a]	Alleles[b]	Phenotype
BW3891	*pho-21*	Constitutive
BW3895	*pho-27*	Constitutive
BW3893	*pho-28*	Constitutive
BW6509	*pho-21 phoR68*	Variable
BW6512	*pho-27 phoR68*	Variable
BW6514	*pho-28 phoR68*	Variable
BW6516	*pho-30*(Am) *phoR68*	Variable

[a] All strains were derived from strain BD792, as described in Table 1.
[b] The *pho-21*, *pho-27*, *pho-28*, and *pho-30*(Am) alleles map in the PST-*phoU* locus.

Effects of *phoR* BAP variation on other studies. The variable BAP character in *phoR* mutant strains may have contributed adversely to results in several studies. Because the osmotic induction of BAP synthesis (16) was reported in the *phoR*-variable MC4100 strain, the effects observed may well have been due to the induction or selection of variants expressing the constitutive state and not due to any actual osmotic control over *phoA* expression. Certainly, the glucose effect observed in complex media in the same study was probably identical to the TYEG induction of the constitutive state, as described here. The small amounts of enzyme made in the absence of osmotic or glucose induction suggest that variants in the negative state were probably used in some experiments (15).

Conflicting data have been reported (or implied) with respect to the effects of *envZ* (*perA*, *tpo*) on BAP expression

in *phoR* mutants. However, the contradictory studies used strains with different BAP phenotypes. Studies suggesting a transcriptional control over *phoA* were done in BAP-variable MC4100 *phoR* mutants (3, 6), and consequently, the reduced transcription observed in *envZ* strains could have been due to inadvertently choosing BAP-negative variants in the experiments or to an effect of *envZ* on the variation. Earlier studies showing that *perA* does not interfere with *phoA* transcription were done, however, in constitutive (stable) XPh4 *phoR* mutants (23). Also, whether procaine affected BAP synthesis at the transcriptional (7) or post-translational (15) level may have been due to strain differences, the inadvertent use of different clonal variants, or an effect of procaine on variation. (Preliminary experiments suggest that *phoR* BAP variation affects transcription, as determined with a *phoA-lacZ* fusion [unpublished results].) The control of BAP synthesis observed in *secA* MC4100 *phoR* mutants (14) may also be partly attributable to BAP clonal variation, especially if particular variants were enriched or induced during the course of the experiment. The latter authors mentioned an unexplained variability in BAP synthesis in their experiments (14); this could have been due to BAP variation.

Summary. Without a better understanding of the underlying molecular mechanisms, it is uncertain whether the strain differences observed here reflect fundamental differences between variable (wild type) and constitutive (stable) *phoR* strains. Perhaps variation occurs in both but at a much reduced frequency and has, therefore, gone undetected in the stable strains. Nevertheless, this novel BAP phenotype has striking implications for the molecular regulation of BAP synthesis, and it cannot be entirely explained by the current model for BAP regulation (21). Further studies of this phenomenon are in progress.

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