COMPLEMENTATION TEST BETWEEN ALKALINE PHOSPHATASE REGULATORY MUTATIONS *phoB* AND *phoRc* IN *ESCHERICHIA COLI*

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ABSTRACT

A *phoRc* and a *phoB* mutation belong to the same complementation group suggesting that there is a single positive control gene for alkaline phosphatase synthesis.

GENETIC evidence suggests that alkaline phosphatase (AP) synthesis in *E. coli* is subject to both negative and positive controls. Wild-type cells synthesize AP at a low level when the growth medium contains excess inorganic phosphate (Pi) and derepress synthesis during Pi starvation (HORIUCHI, HORIUCHI and MIZUNO 1959; TORRIANI 1960). The existence of mutants which synthesize AP constitutively (*phoR*) suggests that synthesis is under negative control (ECHOLS *et al.* 1961). However, there are also mutants which cannot derepress AP in response to Pi starvation, suggesting that synthesis is under positive control. Recessive mutations of this phenotype have been described by GAREN and ECHOLS (1962) and are called *phoRc*. The *phoRc* mutations map between *proC* and *phoR* at 8.8 minutes (KREUZER, PRATT and TORRIANI 1975; NAKATA *et al.* 1971; YAGIL, BRACHA and SILBERSTEIN 1970). Recessive mutations of this phenotype called *phoB* and mapping in the same region have also been described by BRACHA and YAGIL (BRACHA and YAGIL 1973; YAGIL, BRACHA and LIFSHITZ 1975). Strong evidence for a positive control gene at this site comes from the isolation of a deletion of *phoB* by BRICKMAN and BECKWITH (1975) which is also incapable of derepressing AP.

Synthesis of the phosphate binding protein (*phoS* gene product) is also derepressed during Pi starvation by wild-type strains (GAREN and OTSUJI 1964; GERDES and ROSENBERG 1974). The mutants originally designated *phoB* do not derepress phosphate binding protein (WILLSKY and MALAMY 1976). Thus synthesis of AP and of phosphate binding protein appear to be coordinately controlled. However, the mutants originally designated *phoRc* do derepress phosphate binding protein during Pi starvation (GAREN and OTSUJI 1964; WILLSKY and MALAMY 1976; E. YAGIL, personal communication) implying that the mechanisms of control are not identical for the two proteins. This difference between the *phoB* and *phoRc* mutations raises the question of whether there are one or

two positive control genes for AP synthesis located near the negative control gene *phoR*. We have performed a genetic complementation test between a *phoB* mutation and a *phoRc* mutation using an F′ episome. The *phoB* mutant chosen, LEP1, meets the criterion of not synthesizing phosphate binding protein; the *phoRc* mutant S3 does synthesize phosphate binding protein (WILLSKY and MALAMY 1976).

MATERIALS AND METHODS

Strains of *E. coli* K12 are described in Table 1. Media (KREUZER, PRATT and TORRIANI 1975), bacterial mating, acridine orange treatment (PRATT and GALLANT 1972), and P1 transduction (MILLER 1972), have been previously described.

The complementation test was performed as follows: A single colony of each strain was picked from a "121" tris minimal plate and suspended in 0.2 ml 0.85% saline. For the culture in excess Pi 0.05 ml was inoculated in 5 ml "121" medium containing 6.7×10^{-4} M inorganic phosphate and growth limiting lactose or glucose (0.2 mg/ml). For the Pi limited culture (AP is derepressed), 0.05 ml was inoculated in 5 ml "121" medium containing 6.7×10^{-5} M inorganic phosphate and lactose or glucose (2 mg/ml). The cultures were incubated at 37° with agitation for 40 hours. The optical density (540nm) of each culture was measured and the remainder was toluenized (0.05 ml toluene/ml culture) at 37° for 20 minutes. A 0.2 ml sample of the culture or of an appropriate dilution was added to 1.8 ml of substrate mix (0.01 M para-nitrophenol-

TABLE 1

Bacterial strains used

Strain	Genotype	Source or derivation
HD234	*lac str+*/F′*lac+phoA+proC+phoB+ phoR+tsx+*	PRATT and GALLANT 1972
P678	F⁻*thr leu lacY phoA+phoB+phoR+ supE str*	BACHMANN 1972
P678F	*lacY phoA+phoB+phoR+*/F′*lac+ phoA+phoB+phoR+*	HD234 × P678
LEP1	F⁻*lac phoA+proC phoB phoR+ tsx purE trp str*	BRACHA and YAGIL 1973
HL12	*lac phoA+ proC phoB phoR+tsx purE trp*/F′*lac+phoA+proC+phoB phoR+tsx+*	HD234 × LEP1 and homogenotization
S3PV	F⁻*thr leu lacY phoA+phoB+phoRc tsx supE val*^{R}	Valine resistant mutant of recombinant from S3 × P678 (KREUZER, PRATT and TORRIANI 1975)
HL13	*thr leu lacY phoA+phoB+phoRc tsx supE*/F′*lac+phoA+proC+phoB phoR+tsx+*	HL12 × S3PV
HL13L	*thr leu lacY phoA+phoB+phoRc tsx supE*	Lac⁻ clone from acridine orange treatment
HL13R	*thr leu lacY phoA+phoB+phoRc tsx supE tsu1*/F′*lac+phoA+proC+ phoB phoR+tsx+*	Isoleucine-valine independent *tsu1* transductant (P1vir) of HL13 *ilv* by X8605 *tsu1* (GUARENTE and BECKWITH)
HL14	*lac phoA+ proC phoB phoR+ tsx*/F′*lac+ phoA+proC+phoB phoR+tsx+*	HL13 × LEP1

phosphate, 1 M Tris, pH 8.2). The increase in optical density (410 nm) at 37° was followed. One unit of AP activity is defined as an increase in optical density (410 nm) of one per minute. Specific activity is AP activity per ml culture divided by the cell concentration (optical density at 540 nm).

RESULTS AND DISCUSSION

Strain HD234, which contains an F'*lac*$^+$*phoA*$^+$*proC*$^+$*phoB*$^+$*phoR*$^+$*tsx*$^+$ episome, was mated with strain LEP1, which contains a revertible *phoB* mutation (MORRIS *et al.* 1974). A Lac$^+$ Pro$^+$ streptomycin-resistant recombinant capable of derepressing AP was recovered and was irradiated briefly by ultraviolet light (140 ergs/mm^2) to stimulate recombination between episome and chromosome. Lac$^+$ clones incapable of derepressing AP were obtained. One of these which segregated Lac$^-$ colonies upon ultraviolet irradiation and was capable of transferring *lac*$^+$ *tsx*$^+$ was inferred to be homozygous *phoB*/F'*phoB* and designated HL12. HL12 was mated with the *phoRc* strain S3PV. A Lac$^+$ Pur$^+$ Trp$^+$ valine resistant recombinant (HL13) which segregated Lac$^-$ and T6 resistant colonies was inferred to be *phoRc*/F'*phoB*. This partial diploid was tested for complementation between *phoB* and *phoRc* by assaying the AP synthesized during Pi starvation.

The AP specific activity following approximately 24 hours of Pi starvation of the *phoB* strain LEP1 is about 1600-fold less than that of the wild-type F$^-$ P678 (Table 2). The *phoRc* strain S3PV has a specific activity about 300-fold less than wild-type. The introduction of the wild-type F' episome (carrying *phoA*$^+$) from HD234 into the wild-type F$^-$P678 (P678F) causes a fourfold increase in specific activity. The *phoRc*/F'*phoB* partial diploid (HL13) has a specific activity 100-fold less than the wild-type partial diploid (P678F), indicating that full complementation does not occur. HL13 has substantially more activity than either of the mutant F$^-$ strains: 60-fold more than F$^-$*phoB* and 10-fold more than F$^-$*phoRc*. However, the homozygous *phoB*/F'*phoB* partial diploid (HL12) has the same

TABLE 2

Complementation test between phoB *and* phoRc

Strain	Genotype *phoA*	Genotype *phoB-phoRc*	AP specific activity Excess Pi	AP specific activity Limiting Pi
P678	+	+	0.029	6.8
P678F	+/+	+/+	0.082	26.0
S3PV	+	*phoRc*	0.018	0.021
LEP1	+	*phoB*	0.012	0.0042
HL12	+/+	*phoB/phoB*	0.025	0.24
HL13	+/+	*phoRc/phoB*	0.035	0.24
HL13L	+	*phoRc*	0.014	0.018
HL13R	+/+	*phoRc/phoB*	0.024	0.27
HL14	+/+	*phoB/phoB*	0.023	0.19

The complementation test is described in MATERIALS AND METHODS. Specific activity is AP activity per ml culture divided by the optical density of the culture at 540 nm.

derepressed specific activity as *phoRc*/F′*phoB* (HL13), suggesting that the increase in activity is caused by the introduction of the F′*phoA*$^+$*phoB* itself, not by interaction between the *phoB* and *phoRc* mutations. We have no explanation for why *phoA*$^+$*phoB*/F′*phoA*$^+$*phoB* (HL12) should make more AP than F$^-$*phoA*$^+$*phoB* (LEP1). However, these data indicate that there is no complementation between the *phoB* and *phoRc* mutations for AP synthesis.

In order to ensure that HL13 contains F′*phoB* and that a valid complementation test was performed, other possible genotypes have been excluded. HL13 can transfer the F′ episome intact to LEP1 (F$^-$*phoB*). Selection for Thr$^+$ Leu$^+$ Lac$^+$ Pro$^+$ yields a new partial diploid (HL14) which is also T6 sensitive. HL14 segregates Lac$^-$ and T6 resistant colonies implying that it is heterozygous for *lac* and *tsx* which bracket *phoA*, *phoB*, and *phoR*. It is thus unlikely that a part of the F′episome including any of the AP genes has been lost. HL14 should be homozygous *phoB*/F′*phoB* and have the same phenotype as HL12 (*phoB*/F′*phoB*). The derepressed AP specific activity is the same in HL14 and HL12 (Table 2). Thus the possibility that HL13 is homozygous *phoRc*/F′*PhoRc* may be excluded. The presence of the *phoRc* mutation on the chromosome was checked by curing HL13 of its F′episome by acridine orange treatment (HIROTA 1960). The Lac$^-$ cured strain (HL13L) has about the same derepressed AP specific activity as the *phoRc* strain S3PV (Table 2). From these results, the genotype of HL13 is confirmed as *phoRc*/F′*phoB*.

The failure of the *phoRc* and *phoB* mutations to complement implies that there is only a single positive control gene (*phoB*) for AP synthesis. An alternate hypothesis that one of the mutations has a polar effect on a second cistron is unlikely. By this hypothesis, one of the mutations is a nonsense mutation or a frameshift mutation which introduces nonsense, and the resulting premature chain termination prevents expression of the second promoter distal cistron which is the site of the other mutation. The available evidence does not support this hypothesis. *PhoRc* mutation S3 (unpublished experiments, this laboratory) is not suppressed by the amber suppressor gene *tyrT* (*supF*) and the complementation test was done in P678 background which includes the amber suppressor gene *supE*. More conclusive evidence was obtained by introducing a polarity suppressing mutation into HL13. GUARENTE and BECKWITH (in press) have described a mutation (*tsu1*) which appears to map in the *rho* gene coding for a transcription termination factor. They have shown that *tsu1* affects both normal termination and mutational polarity. It is expected to suppress polarity caused by any type of mutation. If the lack of complementation between *phoRc* and *phoB* were the result of one of the mutations being polar, the introduction of the *tsu1* mutation should cause an increase in the expression of the promoter distal cistron and a consequent increase in AP synthesis. However, the presence of *tsu1* (strain HL13R) has no effect on AP synthesis (Table 2).

The function of *phoB* has been studied by *in vitro* synthesis of AP. The *phoB* gene product has a positive effect on transcription from *phoA in vitro* (INOUYE *et al.*, in press). Synthesis of phosphate binding protein, but not of AP, by *phoRc* mutants could be interpreted as a difference between the control sites at *phoS* and

phoA. A *phoRc* mutated positive control factor might be capable of activating transcription at *phoS*, but not at *phoA*. The *phoB* mutants, which include an extensive deletion, do not synthesize phosphate binding protein or AP. Presumably the *phoB* mutated positive control factor cannot activate transcription at either *phoA* or *phoS*.

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