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Complementation Tests Between Alkaline Phosphatase-Constitutive Mutants (*phoS* and *phoT*) of *Escherichia coli*

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Complementation tests between *phoS* and *phoT* mutations showed that they belong to the same cistron. Homozygosis of a heterozygotic partial diploid resulted from allelic transfer from the chromosome to the F′ episome.

Alkaline phosphatase (AP) in *Escherichia coli* is repressed by P_i. *phoS* and *phoT* are two types of mutations which cause the cells to synthesize AP constitutively. *phoS* and *phoT* are associated with the transport of P_i; they are linked at 83 min on the genetic map and are remote from the structural gene (*phoA*) of AP (8.5 min [1]). *phoS* and *phoT* mutants differ from each other by the absence (in *phoS*) or presence (in *phoT*) of a periplasmic P_i-binding protein (9, 19, 21; Fig. 1). At least two P_i transport systems were characterized in *E. coli*, phosphate-specific and P_i transport (14-16). *phoS* and *phoT* belong to the inducible phosphate-specific transport system, and it has been proposed that *phoS* and *phoT* are two cistrons, the former coding for the periplasmic P_i-binding protein and the latter coding for another function in the phosphate-specific transport system (18). However, no critical genetic test has been performed to verify that these two kinds of closely linked and phenotypically similar mutations do indeed belong to two different functional units (cistrons). To test this question, we performed complementation tests between *phoS* and *phoT* mutations.

To study complementation between two recessive mutations in *E. coli*, an F′ factor carrying one mutation can be introduced, by sexduction, into an F^- strain whose chromosome carries the other mutation. If this partial diploid retains the mutant phenotype, the two mutations do not complement each other and probably belong to the same cistron.

An F′ factor carrying a *phoS* or *phoT* mutation can be obtained by a recombinational process called homozygosis, in which F′ *pho*$^+$/F^- *pho* diploids yield spontaneous or UV-induced F′ *pho*/F^- *pho* homozygotic segregants (3, 4). Strain AN862 (all strains used are listed in Table 1) contains the F′111 episome, which extends between *pyrE* (81 min) and *argH* (89 min) on the chromosome, covering *ilv*, *phoS*, and *phoT* (1). This episome was introduced into strain AN346 *phoS86*, and sexductants were plated on unsupplemented minimal medium plates.

These plates contained ample P_i to repress AP synthesis, and only constitutive colonies showed AP activity. When the plates were sprayed for AP activity (19a), about 15% of the colonies showed enzymatic activity. These were considered F′ *phoS86*/F^- *phoS86* homozygotes, and one of them was used as the F′ donor for

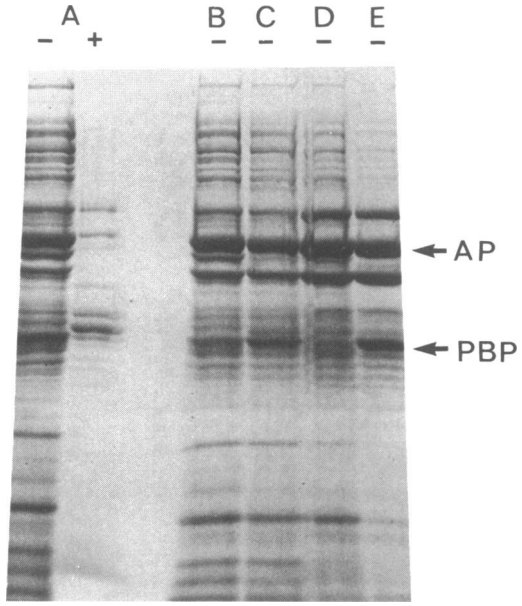

FIG. 1. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of periplasmic proteins grown in excess (+) or in low (−) concentrations of P_i. (A) AN346 (wild type); (B) AN346 phoS86; (C) AN346 phoT90; (D) AN346-16 phoS::Tn5; (E) AN346-20 phoT::Tn5. Methods were as described (21) except that the proteins were heated for 10 min at 100°C in the presence of 1.6% (wt/vol) sodium dodecyl sulfate and 2.8% (vol/vol) β-mercaptoethanol. Purified AP and P_i-binding protein (PBP) were used as markers (arrows). The weak bands at the PBP position in lanes B and D are due to the presence of another protein (19).*

this mutation. Similarly, homozygous F′ donors were obtained for other *phoS* and *phoT* mutations. This process of homozygosis is *recA* dependent because crossing F′111 into strain AN346-25 *phoS86* (carrying *recA*) yields no segregating constitutive colonies though the efficiency of sexduction has not been altered.

Each of the F′ factors constructed with a *phoS* or *phoT* mutation was then introduced back into F^- strains carrying a *pho* mutation, *recA*, and a transposon (Tn*10*) conferring resistance to tetracycline. The Tc^r marker was introduced to counterselect against the F′ donors. The sexductants were plated on minimal medium plates supplemented with tetracycline, and the colonies obtained were sprayed for AP activity. Table 2 shows the results of complementation tests made between two *phoS* (*phoS78* and *phoS86*) and two *phoT* (*phoT90* and *phoT101*) mutations in all possible combinations. The inducible pho^+ recipient strain (AN346-25) was included as a control. All *phoS*/*phoT* heterozygotes were constitutive, indicating lack of complementation. In each case a colony was picked and grown overnight on selective minimal medium (to avoid loss of the F′ factor) with either 1 mM P_i (to repress AP) or 0.0125 mM P_i (condition of AP derepression), and AP was assayed. The quantitative data for AP activity confirmed the qualitative observations made directly on the selective plates.

In the F′ pho^+/F^- *pho* heterozygotes described before, we obtained a high rate (up to 21%) of AP-constitutive segregants. The F′ particle used (F′111) is relatively large, and a recent report has shown that large plasmids can become shorter as a result of deletions, a *recA*-dependent process (13). Therefore, we considered the possibility that the supposedly *pho*/*pho* homozygotes could have resulted from a deletion

TABLE 1. *Bacterial strains*

Strain	Sex	Genotype	Origin or reference
C78	HfrC	*phoS78*	8
C86	HfrC	*phoS86*	8
C90	HfrC	*phoT90*	8
C101	HfrC	*phoT101*	8
CS101B25	HfrC	*srl*::Tn*10 gabC metB recA*	11
AN862	F′111	F′ *pyrE*$^+$ *phoST*$^+$ *ilvC*$^+$ *argH*$^+$/F^- *pyrE ilvC argH purE recA gyrA rpsL*	6
AN346	F^-	*argH ilvC pyrE entA rpsL*	6
AN346	F^-	*ilvC*$^+$ *phoS78* derivative of AN346	Transduced by C78 → AN346, selected for $IlvC^+$, scored for AP constitutivity
AN346	F^-	*ilvC*$^+$ *phoS86* derivative of AN346	Transduced by C86 → AN346, selected for $IlvC^+$, scored for AP constitutivity
AN346	F^-	*ilvC*$^+$ *phoT90* derivative of AN346	Transduced by C90 → AN346, selected for $IlvC^+$, scored for AP constitutivity
AN346	F^-	*ilvC*$^+$ *phoT101* derivative of AN346	Transduced by C101 → AN346, selected for $IlvC^+$, scored for AP constitutivity
AN346-25	F^-	AN346 *srl*::Tn*10 recA*	Transduced by CS101B25 → AN346, selected for Tc^r, scored for UV sensitivity
AN346-25	F^-	AN346 *phoS78 srl*::Tn*10 recA*	Transduced by CS101B25 → AN346 *phoS78*, selected for Tc^r, scored for UV sensitivity
AN346-25	F^-	AN346 *phoS86 srl*::Tn*10 recA*	Transduced by CS101B25 → AN346 *phos86*, selected for Tc^r, scored for UV sensitivity
AN346-25	F^-	AN346 *phoT90 srl*::Tn*10 recA*	Transduced by CS101B25 → AN346 *phoT90*, selected for Tc^r, scored for UV sensitivity
AN346-25	F^-	AN346 *phoT101 srl*::Tn*10 recA*	Transduced by CS101B25 → AN346 *phoT101*, selected for Tc^r, scored for UV sensitivity
594	F^-	*lac rpsL*	F. Stahl
594-16	F^-	594 *phoS*::Tn*5*	Infected 594 with λ::Tn*5*, selected for Km^r, scored for AP constitutivity
594-20	F^-	594 *phoT*::Tn*5*	Infected 594 with λ::Tn*5*, selected for Km^r, scored for AP constitutivity
AN346-16	F^-	AN346 *phoS*::Tn*5*	Transduced 594-16 *phoS*::Tn*5* → AN346, selected for Km^r
AN346-20	F^-	AN346 *phoT*::Tn*5*	Transduced 594-20 *phoT*::Tn*5* → AN346, selected for Km^r

TABLE 2. *Complementation tests between phoS and phoT mutations*[a]

Recipient[b]	Donor[c]			
	F'111 *phoS78/ phoS78* (4.3, 6.6)	F'111 *phoS86/ phoS86* (4.7, 7.9)	F'111 *phoT90/ phoT90* (2.3, 7.1)	F'111 *phoT101/ phoT101* (5.7, 3.2)
AN346-25 (wild type) (0.06, 2.7)	0 (0.04, 4.1)	0 (0.08, 2.5)	0 (0.01, 3.8)	0 (0.05, 5.9)
AN346-25 *phoS78* (3.7, 4.0)	100 (3.8, 6.0)	100 (5.0, 7.0)	100 (2.5, 7.8)	100 (3.1, 4.9)
AN346-25 *phoS86* (2.9, 4.2)	100 (3.7, 6.8)	100 (4.3, 6.7)	100 (2.8, 6.4)	100 (4.2, 5.9)
AN346-25 *phoT90* (2.1, 4.3)	100 (3.9, 5.7)	100 (2.4, 5.6)	100 (2.5, 7.6)	100 (2.1, 5.3)
AN346-25 *phoT101* (6.1, 6.4)	100 (3.4, 5.8)	100 (4.1, 5.4)	100 (4.9, 8.0)	100 (4.2, 6.2)

[a] Numbers show percentage of constitutive sexductant colonies on the selective plates (19a); in each case, at least 60 colonies were counted. From each cross and each parent, one colony was isolated, grown in M9 minimal medium (12) containing 72 mM P_i, washed, and grown overnight in minimal 121 medium (17) containing either 1 mM P_i (high P_i) or 0.0125 mM P_i (low P_i). Each culture was then washed with 0.1 M Tris-hydrochloride buffer (pH 8.0) and treated with 5% toluene, and AP was assayed with 1 mg of *p*-nitrophenylphosphate per ml as previously described (20). The numbers in parentheses show specific enzyme activity (enzyme units/cell density) (first number, cells grown in high P_i; second number, cells grown in low P_i).

[b] All are *recA*.

[c] See text.

of the *phoS-phoT* region in F'111, in which case the conclusion drawn above concerning lack of complementation would become meaningless. To rule out this possibility and to verify that homozygosis is a recombinational transfer of alleles from chromosome to the F' episome, we performed the following experiment. The kanamycin-resistant transposon Tn*5* was "hopped" from bacteriophage lambda into the *phoST* locus (E. Yagil et al., Genetics, in press). Such an insertion yielded cells which were simultaneously constitutive for AP and resistant to kanamycin, both traits cotransducible with *ilvC* (44.8 and 26%, respectively). This Tn*5*-mediated mutagenesis yielded both phenotypes, *phoS* (lacking P_i-binding protein) and *phoT* (containing P_i-binding protein; Fig. 1). The idea was to obtain, by homozygosis, an F'111 carrying one of these constitutive *pho*::Tn*5* mutations (as described before) and to test whether, along with AP constitutivity, the kanamycin resistance (Km^r) trait was transferred onto the F' particle. This would prove that homozygosis is an allelic transfer and that no deletion is involved. F'111 was sexduced into strain AN346 *phoS*::Tn*5*, and an AP-constitutive homozygote segregant was obtained. The F' particle was then transferred into AN346-25 *phoT90*. The resulting sexductants were all constitutive and, in addition, they were all resistant to kanamycin (94 of 94); i.e., without any selection pressure for Km^r, the *phoS*::Tn*5* allele crossed over onto the F' factor, ruling out the possibility of a deletion. In addition, another case of noncomplementation between *phoS*::Tn*5* and *phoT90* was evident.

We have demonstrated that *phoS* and *phoT* are not separate cistrons because, by complementation tests, *phoS* and *phoT* mutations belong to the same functional unit. It should be pointed out that complementation was tested for constitutivity of AP and not for P_i transport. However, these mutants were originally isolated as AP constitutive (7, 8). Garen and Otsuji (8) have found that *phoS* and *phoT* mutations each form a separate group on the genetic map. Suppressible amber mutants are known for both types of mutations (7), indicating that both regions code for a protein (presumably the P_i-binding protein). One possibility is that the *phoS* region codes for a signal sequence (2, 5, 10) which allows the P_i-binding protein to traverse the cytoplasmic membrane to the periplasm. In this case a larger precursor ought to be found trapped in the cytoplasm or in the cytoplasmic membrane of *phoS* strains. A second expectation is that the P_i-binding protein present in *phoT* mutants should show no P_i-binding activity. However, our suggestion is somewhat contradicted by the existence of a *phoT*::Tn*5* mutation (Fig. 1) or suppressible *phoT* amber mutants, both containing unaltered P_i-binding protein as detectable on gels. Finally, a third type of mu-

tants in the phosphate-specific transport system, known as *pst*, are also constitutive for AP (19). These ought to undergo similar complementation tests with *phoS* and *phoT* before being assigned as a separate cistron.

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