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Short Communication

GENETIC LOCATION OF THE *phr* GENE OF *Escherichia coli* K-12

DAVID A. YOUNGS * and KENDRIC C. SMITH **

Department of Radiology, Stanford University School of Medicine, Stanford, CA 94305 (U.S.A.)

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Our transductional mapping data locate the *phr* gene between *kdp* and *gltA*, i.e., at ~15.9 min on the *Escherichia coli* chromosome. This location was confirmed using strains carrying deletions in this region.

Photoreactivation is defined as the reversal of ultraviolet-induced killing by a subsequent exposure to near-UV and/or visible light (for a review see ref. 6). A *phr* mutant of *E. coli* B that lacks active photoreactivating enzyme was isolated by Harm and Hillebrandt [4]. This mutation was roughly mapped by Van de Putte et al. [9]. Their results located the *phr* gene near *gal* on the *E. coli* chromosome. A more careful mapping of the *phr* locus was not reported until the studies by Sutherland et al. [8], using strains with various deletions in the *gal* region. These workers found that strains SA206 and SA244, which had deletions extending from *chlA* to *gal*, were Phr⁻. However, two strains with deletions extending from *chlA* to *att*λ were Phr⁺. The conclusion of Sutherland et al. [8] from these results was that the *phr* locus is probably between *gal* and *att*λ.

In our efforts to genetically manipulate the *phr* mutation we made the following observations that are the subject of this report: (i) all of the strains that we examined with deletions in the *gal-att*λ region, including SA206 and SA244, were Phr⁺. (ii) Transductional mapping studies indicate that the *phr* gene lies between *kdp* and *gltA*. (iii) Strains with deletions between *kdp* and *gltA* are Phr⁻. These results contradict the findings of Sutherland et al. [8].

The first indication that *phr* might not be located between *gal* and *att*λ resulted from our unsuccessful attempt to use SA291 Δ(*gal-chlA*) as a Phr⁻ donor in strain-construction experiments. This strain proved to be Phr⁺. A number of other strains with deletions extending through the *gal-att*λ region were also checked. These included strains RW361, KS302, SA242, 901, SA206 and SA244 (see Table 1 for the characteristics of these strains). All were Phr⁺. Two known *phr* strains were also checked, Hs30 and DY314 (Table 1). These strains

* Present address: Baxter/Travenal Laboratories, 6301 N. Lincoln Ave., Morton Grove, IL 60053 (U.S.A.)

** Please address reprint requests to this author.

TABLE 1

BACTERIAL STRAINS

Our stock number	Source number	Genotype	Source
SR336	901	Δ(*galE*-λ_Q) Δ(*uvrB-chlA*)	A.M. Campbell
SR337-339	SA206	Δ(*gal-chlA*)	A.M. Campbell, B.M. Sutherland and S. Adhya
SR340	SA244	Δ(*gal-chlA*)	S. Adhya
SR341, 342	SA291	Δ(*gal-chlA*) *his*	A.M. Campbell A.K. Ganesan
SR343	SA242	Δ(*gal*-λ_E) *his str*	A.M. Campbell
SR344	KS302	Δ(*gal-uvrB*)	A.M. Campbell
SR345	RW361	Δ(*gal-bio*)	J.D. Gross
SR346	DD45	F^- *gal bioA leu thi chlA str*	A.M. Campbell
SR347	X9170	F^- *kdp gltA gal*	W. Epstein
SR348	Hs30 [b,c]	*phr uvrB*	R.M. Tyrrell
SR114	AB1886	F^- *uvrA6 arg pro his ara gal lac xyl mtl str tsx thr leu thi*	S. Linn
SR248	KH21	F^- *leuB bio rha lacZ str thyA thyR metE malB*	R.B. Helling
SR349	DY292	F^- *leuB bio rha lacZ str thyA thyR metE uvrA6*	P_1 · AB1886 × KH21, select Mal^+
SR350	DY314 [c]	F^- *leuB rha lacZ str thyA thyR metE uvrA6 uvrB phr*	P_1 · Hs30 × KH21, select Bio^+
SR351	DY324	F^- *phr*	P_1 · DY314 × X9170, select Gal^+
SR369	TK2063 [d]	F^- *thi rha* Δ(*gal-bio*) *trkD1 trkA405*	W. Epstein
SR370	TK2063-320	TK2063 with λ in *kdpD*	W. Epstein
SR371	A1 [d]	derived from TK2063-320 and contain a deletion from *kdp* through at least *gltA*	W. Epstein
SR372	A4 [d]	derived from TK2063-320 and contain a deletion from *kdp* through at least *gltA*	W. Epstein
SR373	103 [d]	derived from TK2063-320 and contain deletions from *kdp* but not extending through *gltA*	W. Epstein
SR374	107	derived from TK2063-320 and contain deletions from *kdp* but not extending through *gltA*	W. Epstein
SR375	213	derived from TK2063-320 and contain deletions from *kdp* but not extending through *gltA*	W. Epstein
SR376	214	derived from TK2063-320 and contain deletions from *kdp* but not extending through *gltA*	W. Epstein
SR377	230	derived from TK2063-320 and contain deletions from *kdp* but not extending through *gltA*	W. Epstein
SR378	235	derived from TK2063-320 and contain deletions from *kdp* but not extending through *gltA*	W. Epstein

[a] Symbols are those used by Bachmann et al. [1].

[b] Strain Hs30 [5] is a Uvr^- derivative of the *phr* mutant of *E. coli* B described by Harm and Hillebrandt [4].

[c] The cotransduction data (Table 2) indicate that DY314 contains both the *uvrB* and *phr* alleles. Strain Hs30 was the donor of both of these markers in the construction of DY314.

[d] Deletions in the region of *phr* were isolated by D.B. Rhoads. Bacteriophage λ*c*I857 was integrated into the *kdp* genes of strain TK2063 by a slight modification of the method of Shimada et al. [7], using two cycles of penicillin selection in medium containing approximately 1 mM K^+ (L.A. Laimins, D.B. Rhoads, and W. Epstein, in preparation). A strain with the phage in *kdpD*, TK2063-320, was used to select spontaneous deletions as derivatives able to grow at 42°C. The survivors were scored for recombination with several *kdp* mutants, and for growth on minimal medium. All 8 deletions studied here extend past the most *kdpD* distal *kdp* mutation available, *kdpA10*. Two of the deletions, A1 and A4, require glutamate or proline for growth and therefore harbor deletions extending into *gltA* or beyond. The strains were maintained and grown on KML medium [3].

carry the *phr* mutation originally isolated in *E. coli* B by Harm and Hillebrandt [4]. These strains are Phr^-. These results were based upon full survival curves using log-phase cells grown in LB broth [10], resuspended in buffer (5.8 g Na_2HPO_4 and 3.5 g KH_2PO_4 per liter) and UV-irradiated (254 nm). The irradiated cells were diluted and plated on YENB agar (7.5 g Difco yeast extract and 23 g Difco nutrient agar per liter) either immediately, or after a 20 min exposure to photoreactivating light [12].

Since our results appeared in conflict with those of Sutherland et al. [8], we tried to duplicate their experimental conditions using three different samples of strain SA206 (see Table 1). These samples were Phr$^+$ under all experimental conditions used (e.g., log or stationary, complex or minimal media). In addition, we found no differences in the kinetics of photoreactivation for SA206 and a *uvrB5* strain (data not shown). We are at a loss to explain why strain SA206 appears to be Phr$^+$ in the present experiments but Phr$^-$ previously [8]. However, Dr. Sutherland now finds that strain SA206 shows about a 10-fold increase in survival after photoreactivation from an initial survival of 10^{-3} (personal communication), whereas we observe greater than a 100-fold photoreactivation at the same initial survival level. In either case, strain SA206 cannot be considered to be Phr$^-$.

Our studies indicate that the *phr* gene does not lie in the *gal-att*λ region as previously indicated by Sutherland et al. [8]; yet the conjugational mapping studies of Van de Putte et al. [9] locate *phr* near *gal.* To obtain more exact knowledge of the *phr* locus, we initiated a series of transductional mapping experiments.

The *phr* mutation of Hs30 was first transduced into the K-12 strain, DY292. Approximately 2—3% cotransducibility between *phr* and *bio* was observed. A *phr uvrB* transductant from this cross (DY314) was used as the donor in the next preliminary cross with strain DD45. Of 100 Gal$^+$ transductants checked from this cross, 8 were Phr$^-$, 40 were Uvr$^-$ and none were Uvr$^-$Phr$^-$. These

TABLE 2

COTRANSDUCTION OF *phr* WITH *kdp, gltA, gal* AND *uvrB* [a]

Selected marker	Unselected marker				
	Kdp$^+$	Phr$^-$	Glt$^+$	Gal$^+$	Uvr$^-$
Glt$^+$	181/270	199/270	—	62/270	0/60
Kdp$^+$	—	40/55	38/55	9/55	0/27
Gal$^+$	51/238	66/238	86/238	—	32/78
Kdp$^+$Glt$^+$	—	214/219	—	54/219	0/53
Kdp$^+$Gal$^+$	—	55/60	60/60	—	1/18
Glt$^+$Gal$^+$	96/148	105/148	—	—	3/40

[a] The recipient strain was X9170 *kdp gltA gal*, and the P_1 donor strain was either DY314 *phr uvrB* or DY324 *phr*. The transduction procedure was similar to that described by Youngs and Bernstein [10], except that the sodium citrate step was omited. The media used were: LB broth [10] MM agar [11], KO agar [2], and YENB agar. Glutamate (2 mM) was included in media used for growth of Glt$^-$ strains. The Kdp$^-$ phenotype was scored as the inability to grow on KO agar [2]. The Glt$^-$ phenotype was scored as the inability to grow on glucose MM agar in the absence of glutamate. The Gal$^-$ phenotype is indicated by the lack of growth on galactose MM agar. The Phr$^+$, Phr$^-$, and also Uvr$^+$, Uvr$^-$ phenotypes were distinguished in the following manner: cells were suspended in buffer and ~0.01 ml samples were placed on a series of YENB agar plates. Pairs of these plates were exposed to a given UV fluence and one plate from each pair was then exposed to photoreactivating light [12] for 5 min. The series of plates was then incubated at 37°C to determine the relative cell survival. The numbers represent the total number of transductants receiving the unselected marker (numerator), and the total number screened (denominator).

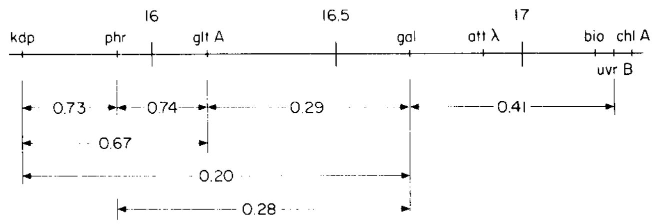

Fig. 1. Order of genetic loci in the *kdp-chlA* region of the *E. coli* chromosome. The average frequency of cotransduction between markers is shown (calculated from the data in Table 2). The position of each of the genes except *phr* is from Bachmann et al. [1]. Our transductional mapping data indicate that the *phr* gene is located between *kdp* and *gltA*, at about 15.9 min on the *E. coli* chromosome.

two experiments and the known map order of *gal*, *bio* and *uvrB* [1] suggested that the order for these loci is *phr gal bio uvrB*.

For more careful mapping, strain X9170 *kdp gltA gal* was used as the recipient in a cross with DY314 *phr uvrB* or DY324 *phr*. The results from a number of transduction experiments of this type are given in Table 2. The order *kdp phr gltA gal* is indicated by the analysis of each class of transductants. For example, of 238 transductants selected for the Gal$^+$ phenotype, 86 were Glt$^+$, 66 were Phr$^-$, and 51 were Kdp$^+$. This orientation is also indicated by the fact that 97.7% of the Glt$^+$ Kdp$^+$ transductants were Phr$^-$, while only 70.9% of the Glt$^+$ Gal$^+$ transductants were Phr$^-$.

The average cotransduction frequencies between the various genes were calculated from the results in Table 2, and are shown in Fig. 1. The *phr* gene is located approximately midway between *kdp* and *gltA*, i.e., at about 15.9 min on the *E. coli* chromosome.

Our mapping data were then confirmed using a series of strains (derivatives of TK2063) containing deletions running from *kdp* towards and sometimes including *gltA* (see Table 1), and testing them for photoreactivation. Cells were grown overnight in KML medium [3], UV irradiated in phosphate buffer at $\sim 2 \times 10^8$ cells per ml with 125 Jm^{-2}, giving a survival on KML plates before photoreactivation of $\sim 6 \times 10^{-3}$. The experiment was run twice. The A1 and A4 strains, carrying deletions extending from *kdp* to *gltA* or beyond were Phr$^-$, as were strains 107, 214, and 235. The deletions in the latter strains do not extend through *gltA*. Strains TK2063, TK2063-320, 103, 213 and 230 were Phr$^+$. The deletions in the last three strains do not extend through *gltA*.

It is of interest that these strains also carry a deletion between *gal* and *bio* and therefore should all have been Phr$^-$ according to the mapping of Sutherland et al. [8], instead only those that carried additional deletions between *kdp* and *gltA* where Phr$^-$.

In summary, the survival results indicate that strains deleted in the region extending from *gal* to *att*λ are Phr$^+$, in contrast to the results of Sutherland et al. [8]. Our transductional mapping data indicate that the *phr* gene lies between *kdp* and *gltA*, i.e., at ~15.9 min on the *E. coli* chromosome. Our mapping data are confirmed by the observation that strains deleted between *kdp* and *gltA* are Phr$^-$.

A. Sancar and C.S. Rupert (personal communication) have independently obtained results similar to ours. Their mapping data obtained through conjugation, F′ transfer, transduction, and deletion analysis place the *phr* gene to the left of (counterclockwise from) *gal*, and between 15.5 and 15.9 min on the *E. coli* K-12 chromosome.

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