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Mapping of the *pin* Locus Coding for a Site-Specific Recombinase That Causes Flagellar-Phase Variation in *Escherichia coli* K-12

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Although the *vh2* mutation almost entirely prevents phase variation in *Salmonella* spp., an *Escherichia coli* strain that carried the *Salmonella H1* and *H2* region, including the *vh2* mutation, showed phase variation. From this strain, EJ1076, a number of mutants defective in phase variation were isolated, and the symbol *pin* was assigned to their mutant gene. The *pin* locus was mapped between *purB* and *trp* near *purB* by interrupted matings using Tn*10* sites inserted near *pin*. The locus was cotransduced with *purB* by P1 *vir* at a frequency of around 0.33. All the mutations tested were clustered at this locus. Three *E. coli* K-12 strains probably derived via different lines from the wild type have been tested for the presence of *pin*$^+$ by introducing the two Salmonella *H* regions; two were *pin*$^+$, and one was a *pin* mutant.

Salmonella phase variation, first reported by Andrews (2), is the phenomenon that results from the alternate expression, at a definite rate per bacterium per division, of two kinds of antigenically distinct flagella called phase 1 and phase 2 (27). Phase 1 and phase 2 flagella are specified by the respective flagellin genes, *H1* and *H2* (19), which are located at 41 and around 59 min, respectively, on the *Salmonella typhimurium* linkage map (23). The *H2* region is composed of PD (phase determinant) (*vh2* or *hin*)-*H2-rh1*. The product of *vh*2 (variegator of *H2*) (11), which seems to be identical with *hin* (*H2* inversion) (25), causes reversible inversions of the PD segment (7), which has inverted repeats at both ends and includes *vh2* or *hin* as well as a promoter sequence (29) for the cotranscribed *H2* and *rh1* (repressor of *H1*) genes. In one of the two possible orientations of the PD segment, *H2* and *rh1* are expressed, and the product of *rh1* represses *H1* (8, 26), resulting in the phase 2 state. In the other orientation of PD, *H2* and *rh1* are not expressed owing to the reverse orientation of promoter, and consequently only *H1* is expressed, resulting in the phase 1 state.

Escherichia coli K-12 strains that were transduced by phage P1 with *Salmonella H1-i* (antigen *i*) at *hag* (*E. coli* flagellin gene) and the *H2-e,n,x* (antigen *e,n,x*) region at the site corresponding to that in *S. typhimurium* were found to show phase variation between *i* and *e,n,x* (7). When the *vh2*$^+$ *H2 rh1*$^+$ region was transduced, the rate of phase variation was of the order of 10^{-3} per bacterium per division and about 1 order greater than that of the *Salmonella* strain from which the *H2* region was derived. The *vh2 H2 rh1*$^+$ region does not allow phase variation in *S. typhimurium*, but when it was transduced, a rate of 10^{-4} to 10^{-3} per bacterium per division was observed. This region became inactive again when transduced back to *S. typhimurium*. Similar results were obtained with the F′ factors carrying *vh2*$^+$ *H2-e,n,x rh1*$^+$ (EJF56) or *vh2 H2-e,n,x rh1*$^+$ (EJF57) (K. Oosawa, M.S. thesis, Okayama University, Okayama, Japan, 1980). The merodiploids *H1-i*/EJF56 and *H1-i*/EJF57, made by introducing F′ factors into the *H1-i* transductant of *E. coli* (EJ1118), showed phase variation between *i* and *e,n,x*. On the other hand, when EJF57 was introduced to the *Salmonella* monophasic *H1-b vh2 H2-1.2 rh1*$^+$ strain (SJ2503), which expresses only *b*, the resultant merodiploid did not show phase variation, whereas the merodiploid containing EJF56 did show variation between *b* and *e,n,x*, although variation between *b* and *1.2* was not observed. These results suggest that a gene which is able to compensate for the *vh2* mutation may be present in *E. coli* and absent from *Salmonella* spp. This paper deals with isolation and characterization of *E. coli* mutants that lack the ability to suppress the *vh2* mutation. The gene was named *pin* (PD inversion) and was mapped at about 26 min, near *purB*, on the *E. coli* linkage map (4).

MATERIALS AND METHODS

Bacterial strains and phages. All the bacterial strains used were derivatives of *E. coli* K-12 (Table 1). Strain EJ350 is a motile derivative of PA309 (3) that was found to be *pin*$^+$, capable of undergoing phase variation. EJ1076 and EJ1346 were constructed from EJ350 by transducing *Salmonella H1-i* and introducing a *Salmonella H2* region by using Hfr strains derived from *H2* transductants of *E. coli* (7). H680 was found to be a naturally occurring *pin* mutant, and the number *pin-101* was assigned to it. P1CM *clr*100 (22), used for lysogenization, was supplied by K. Kutsukake, Faculty of Science, Tokyo University. For transduction, P1 *vir* was used throughout the experiments to avoid lysogenization since a *din* gene of P1 (18), similar to *gin* of phage Mu (1, 5), can catalyze inversions of the PD segment of *Salmonella vh2* strains (17). λNK173 (*b*221 *rex*173::Tn*10* *c*I857) (12) was supplied by Y. Komeda, National Institute of Genetics.

Media. TLY broth contained 10 g of tryptone (Difco Laboratories), 5 g of Lab Lemco powder (Oxoid Ltd.), 5 g of yeast extract (Difco), and 5 g of NaCl per liter of deionized water. Nutrient agar (NA), nutrient semisolid agar (NSS), and soft agar contained 1.2, 0.3, and 0.5% agar (Wako Pure Chemical Industries), respectively, in E broth that contained 10 g of peptone (Kyokuto Pharmaceutical Co.), 5 g of beef extract (Kyokuto), and 5 g of NaCl per liter of deionized water. Minimal agar was composed of M9 (21) and 1.2% agar, and to it were added, when required, amino acids, thiamine, and nucleic acid bases at concentrations of 20, 1, and 40 mg per liter, respectively. Buffered saline contained 0.85% NaCl in a 1/10 concentration of M9 salts. Streptomycin (Banyu Pharmaceutical Co.), chloramphenicol (Sigma Chemical Co.), and tetracycline (Sigma) were used at concentrations of 200, 12.5, and 25 mg per liter, respectively. Anti-λ serum and anti-flagellum sera were prepared in this laboratory. Anti-flagellum sera made against *Salmonella* monophasic strains were used for *H* derivatives of *E. coli* without absorption by *Salmonella* somatic antigens.

TABLE 1. Bacterial strains used

Strain	Relevant markers[a]	Source or parent
EJ350	*thr-1 leu-6 trp-1 his-1 gal-6 str-9*	PA309 (3) via Y. Hirota
EJ1076	*H1-i vh2 H2-e,n,x rh1*$^+$	EJ350 introduced *Salmonella H* regions
EJ1346	*H1-i vh2*$^+$ *H2-e,n,x rh1*$^+$	Same
EJ1348	*pyrC46 trp*	YK4118 (Y. Komeda [15]) made *trp*
EJ1352	*H1-i vh2 H2-e,n,x rh1*$^+$ *pin-101 purB51*	H680 introduced *Salmonella H* regions
EJ1365	*H1-i vh2 H2-e,n,x rh1 pin-8*	EJ1076
EJ1368	*H1-i vh2 H2-e,n,x rh1 pin-16*	EJ1076
EJ1369	*H1-i vh2 H2-e,n,x rh1 pin-20*	EJ1076
EJ1396	*H1-i vh2 H2-e,n,x rh1 zcg-2*::Tn*10*	EJ1365 cotransduced *pin*$^+$ and Tn*10*
EJ1397	*H1-i vh2 H2-e,n,x rh1 zcg-3*::Tn*10*	Same
EJ1398	*H1-i vh2 H2-e,n,x rh1 pin-8 zcg-2*::Tn*10*	EJ1365 transduced Tn*10*
EJ1399	*H1-i vh2 H2-e,n,x rh1 pin-8 zcg-3*::Tn*10*	Same
EJ1419	*H1-i vh2 H2-e,n,x rh1 purB51*	EJ1352 made *pin*$^+$
H680	*purB51 trp-45*	CSGC5038 (P. de Haan) via Y. Komeda
HfrH	0-*thr*$^+$-*leu*$^+$-*gal*$^+$ . . .	
HfrKL99	0-*pyrC*$^+$-*purB*$^+$-*trp*$^+$ . . .	

[a] Abbreviations for *S. typhimurium* (23) are used for flagellin genes and their regulatory genes. Flagellin genes were used without a superscript plus or minus to avoid confusion of terms in phase variation.

Isolation of *pin* mutants. When strain EJ1076 is inoculated at the center of an NSS plate containing anti-*i* serum and incubated overnight at 37°C, motile bacteria expressing *e,n,x* spread into the medium to form a round swarm. After longer incubation, however, a few fast-spreading swarms appear jutting out from the margin of the ordinary round swarm. These swarms seem to arise from mutations that allow the expression of *e,n,x* predominantly. Two sorts of mutation are considered; one is an *H1* mutation causing the lack or the antigenic change of phase 1 flagella, and the other is a mutation in the gene that controls the rate of phase variation, causing flagellar expression to be fixed at either *H1* or *H2*. Based on this assumption, a number of fast-spreading swarms were isolated on NSS containing anti-*i* serum, tested as to whether their antigens were fixed at *e,n,x*, and further tested for restoration of phase variation by lysogenization with P1CM *clr*100.

Insertion of Tn*10* into sites near *pin*. Two steps were adopted from the method of Kleckner et al. (12–14). (i) Random insertion of Tn*10* into *pin*$^+$ bacteria and P1 *vir* propagation on the resultant Tn*10* transposons. Ten milliliters of TLY broth culture (ca. 5×10^8 cells per ml) of the *pin*$^+$ strain EJ1076 was centrifuged and suspended in 2 ml of TLY broth, and to it was added λNK173 carrying Tn*10* at a multiplicity of infection of <0.1. After adsorption for 30 min at 30°C, 0.1 ml of anti-λ serum was added since the strain used is so sensitive to λ that a large number of tetracycline-resistant (Tet^r) colonies cannot be produced by the ordinary method. The mixture was further incubated for 40 to 50 min at 30°C, and 0.5 ml per plate was spread on NA plates containing tetracycline. The plates were incubated overnight at 37°C. When more than 500 Tet^r colonies appeared per plate, they were harvested with buffered saline while they were still small. The suspension was washed twice, and a part of it was inoculated into 5 ml of broth containing tetracycline and 0.25 ml of anti-λ serum. The broth was then incubated for 1 h at 37°C. P1 *vir* was propagated by the double-agar layer method with this culture and soft agar containing 1/20 of a volume of anti-λ serum. The lysate contained P1 *vir* and λ at concentrations of 2×10^{10} and 3×10^6 PFU/ml, respectively. (ii) Cotransduction of *pin*$^+$ and Tn*10* into *pin* mutants. P1 *vir* thus

propagated was applied to the broth culture of a *pin* mutant at a multiplicity of infection of 0.05 to 0.1. One-half of a milliliter of the mixture was taken into a plate that contained cooled but not solidified NSS containing tetracycline and anti-*e,n,x* serum for the recipient flagellar antigen and was quickly mixed. After solidification, the plates were incubated for 30 h at 37°C. When swarms were detected among a number of nonmotile colonies, they were restreaked onto NA containing tetracycline, and resultant single colonies were stabbed into the two NSS plates containing anti-*i* or anti-*e,n,x* serum. Clones that formed swarms on both plates were isolated as candidates for *pin*$^+$ Tn*10* cotransductants.

Test of *pin*. The presence of *pin*$^+$ in *vh2* mutants was determined by phase variation tests. Swarms in NSS containing anti-*H* serum for phase 1 or phase 2 were stabbed (directly or after reisolation as single colonies) into NSS containing anti-*H* serum for the opposite phase and into NSS containing both antisera. Clones whose motility was not hindered by either antiserum but was hindered by both sera were judged *pin*$^+$. The method for measuring the rate of phase variation has been described (7).

Hfr crosses. Matings were carried out by standard methods (9). The mating interruption was performed by blending 10-fold dilutions for 30 s with a Micronizer (Nihon Seiki). Samples at each interrupted time were diluted 100 times into cooled buffered saline, and an appropriate volume (0.05 to 0.2 ml) was layered with plain agar on selective plates. Tet^r recombinants were selected on NA containing tetracycline and streptomycin, and other recombinants were selected on minimal agar.

RESULTS

Isolation and characterization of *pin* mutants. Ninety-eight fast-spreading swarms were isolated on NSS containing anti-*i* serum from strain EJ1076. Single colonies from them were stabbed into three NSS media containing anti-*i*, anti-*e,n,x*, or both. Twenty-three expressed only *e,n,x*. To examine whether the lack of *i* expression was due to mutation in *H1* or in a gene controlling phase variation, each clone was lysogenized with P1CM *clr*100. Chloramphenicol-resistant (Cm^r) colonies were stabbed into the three NSS media as mentioned above. All the Cm^r colonies from 23 clones formed swarms in NSS containing anti-*i* or anti-*e,n,x* but not in NSS containing both sera, indicating that the mutations are complemented by P1 *din*$^+$ and consequently that the mutants have normal *H1-i* which is repressed owing to the fixed expression of *H2-e,n,x* and *rh1*$^+$. They were considered to be mutants that were unable to suppress *vh2*. The symbol *pin* (PD inversion) was given to the mutant gene. To test the stability of *pin* mutants, the broth cultures started from single colonies of each mutant were centrifuged, and the sediments were smeared as lines on NSS medium containing anti-*e,n,x* serum and were incubated for more than 1 day at 37°C. Out of 23 mutants, 6 produced a number of revertants (10 to 30 swarms per line) which were considered Pin$^+$ since all the revertants tested showed phase variation between *i* and *e,n,x*. The rest produced a few swarms which did not show phase variation but were fixed at *i*; these were probably produced by leakiness of the mutation in either *vh2* or *pin*. The three mutants of the latter group, *pin-8*, *pin-16*, and *pin-20*, were used for further experiments. Rates of variation of P1CM *clr*100 lysogens of *pin-8*, *pin-16*, and *pin-20* from phase 2 to phase 1 were 1.5×10^{-2}, 5.4×10^{-2}, and 1.2×10^{-2} per bacterium per division, respectively, and those of nonlysogens were less than 5.4×10^{-5} per bacterium per division; that is, variation was not detected. Parental EJ1076 showed rates of 8.5×10^{-3} and 9.8×10^{-4} per bacterium per division in the lysogenic and nonlysogenic states, respectively. The rate was 10 times higher in the lysogens than in the nonlysogen EJ1076 and much higher in the lysogens of the *pin* mutants. The mutants were not different from the parent in nutrient requirements, colonial types on NA and minimal agar, or growth rates in TLY broth.

Insertion of Tn*10* near *pin*. Tn*10* (Tet^r) inserted in sites near *pin* was used as a marker for mapping the *pin* locus. The P1 *vir* lysate, propagated on the *pin*$^+$ strain EJ1076 in which Tn*10* was randomly inserted, was applied to strain EJ1365 (*pin-8*), and Tet^r *pin*$^+$ cotransductants were isolated. For five cotransductants isolated, cotransduction frequencies of Tet^r and *pin*$^+$ were about 20% (one cotransductant) and 100% (the rest) when they were transduced again into EJ1365 and Tet^r was selected. They were tested and shown not to be lysogenized with P1. The Tn*10*-insertion sites in the five cotransductants were inferred to be nonessential regions since they showed the same nutrient requirements and growth rate as the parent. EJ1396 and EJ1397, two transductants which showed ca. 20 and 100% cotransduction frequencies, respectively, were used as representatives for further experiments. The sites at which Tn*10* was inserted in EJ1396 and EJ1397 were named *zcg-2*::Tn*10* and *zcg-3*::Tn*10*, respectively (6, 10). The sites were revealed afterwards to be at around 26 min on the linkage map (4).

With strains EJ1396 and EJ1397 as donors and *pin* mutants as recipients, P1 *vir* transduction was carried out to see whether *pin* mutations were clustered. Tet^r transductants obtained were tested for the presence of *pin*$^+$. The results with the three representatives are shown in Table 2. The cotransduction frequencies of *pin*$^+$ alleles with *zcg-2*::Tn*10* were 15 to 25%, and those with *zcg-3*::Tn*10* were 99 to 100%. With the other seven *pin* mutants, cotransduction frequencies of about 100% were also obtained

TABLE 2. Cotransduction between Tn*10* (Tet^r) and *pin*[a]

Donor	Recipient	No. of Tet^r colonies tested	No. of donor *pin* type	Cotransduction (%)
EJ1396 (*pin*$^+$ *zcg-2*::Tn*10*)	*pin-8*	228	35	15.4
	pin-16	90	22	24.4
	pin-20	120	27	22.5
EJ1397 (*pin*$^+$ *zcg-3*::Tn*10*)	*pin-8*	85	84	98.8
	pin-16	121	121	100.0
	pin-20	153	153	99.3
EJ1398 (*pin-8 zcg-2*::Tn*10*)	*pin*$^+$	228	90	39.5
EJ1399 (*pin-8 zcg-3*::Tn*10*)	*pin*$^+$	134	121	90.3

[a] Tet^r transductants selected on NA containing tetracycline were tested for the presence or absence of *pin*$^+$ by stabbing in NSS containing tetracycline and anti-*H* serum. Recipients used were EJ1076 (*pin*$^+$), EJ1365 (*pin-8*), EJ1368 (*pin-16*), and EJ1369 (*pin-20*).

between *pin*$^+$ alleles and *zcg-3*::Tn*10*. The two Tet^r transductants *pin-8 zcg-2*::Tn*10* (EJ1398) and *pin-8 zcg-3*::Tn*10* (EJ1399) were used as donors and were transduced to the *pin*$^+$ strain (Table 2, last two lines). The former showed a significantly higher cotransduction frequency (39.5%), and the latter showed a lower one (90.3%), than when *pin-8* was used as the recipient. The reason for the difference is not clear but will be discussed later. It was inferred that *pin* mutations, at least the 10 mutations tested, clustered in one region, although it remains unknown whether they are in one cistron or not. When *pin-8 zcg-3*::Tn*10* was transduced to the *vh2*$^+$ strain EJ1346, 101 Tet^r transductants tested all showed phase variation, demonstrating that *vh2*$^+$ and *pin*$^+$ compensate for each other. *pin*$^+$ or *pin* mutations were easily transduced by means of *zcg-3*::Tn*10*.

Location of Tn*10* insertion sites by interrupted matings. The region including *pin*$^+$ *zcg-2*::Tn*10* or *pin*$^+$ *zcg-3*::Tn*10* was transduced to HfrH from strain EJ1396 or EJ1397, respectively. The HfrH derivatives carrying Tn*10* were confirmed to have *pin*$^+$ near the Tn*10* insertion site by spot tests on the *pin-8* mutant; *zcg-2*::Tn*10* and *zcg-3*::Tn*10* derivatives produced Tet^r *pin*$^+$ recombinants at frequencies of 35% (9 *pin*$^+$ among 26 Tet^r recombinants tested) and 100% (13 *pin*$^+$ among 13 Tet^r), respectively. These two HfrH derivatives were crossed to the multi-auxotrophic strain EJ350, and the Tn*10* insertion sites were determined by interrupted matings. Tet^r entered after Gal$^+$ and before Trp$^+$. The two Tn*10*-insertion sites were transduced to HfrKL99. The interrupted matings between the KL99 derivative and a *pyrC trp* strain (EJ1348) indicated that Tet^r entered after PyrC$^+$. Similar experiments with a *purB trp* strain (EJ1349) revealed that *zcg-3*::Tn*10* entered immediately after *purB*$^+$ and *zcg-2*::Tn*10* entered a little after *purB*$^+$: the order *purB–zcg-3*::Tn*10–zcg-2*::Tn*10–trp* was inferred.

Mapping of *pin* by cotransduction. Preliminary cotransduction tests between *zcg-3*::Tn*10* and auxotrophic markers revealed that Tet^r *purB*$^+$ was cotransducible, although Tet^r *pyrC*$^+$ and Tet^r *trp*$^+$ were not. The loci of *pin*, *zcg-2*::Tn*10*, and *zcg-3*::Tn*10* were determined by cotransduction frequencies with *purB*. First, P1 *vir* cotransduction between *purB*$^+$ and Tet^r was carried out by using the two Tet^r strains EJ1396 and EJ1397 as donors and the *purB* mutant H680 as the recipient. *purB*$^+$ transductants selected were inoculated on the same selective medium, and after incubation they were replicated by replica plating on NA containing tetracycline. *zcg-2*::Tn*10* and *zcg-3*::Tn*10* were cotransduced with *purB*$^+$ at frequencies of 28 and 47%, respectively (Table 3). When Tet^r was used as the selective marker, however, both donors showed nearly 100% cotransduction frequency of Tet^r and *purB*$^+$; 390 Tet^r *purB*$^+$ among 395 Tet^r tested in the case of *zcg-2*::Tn*10*, and 397 Tet^r *purB*$^+$ among 398 Tet^r tested in the case of *zcg-3*::Tn*10*. This is a strange phenomenon, and it will be discussed. Next, cotransduction between *purB* and *pin* was attempted. Transduction from EJ1076 (*purB*$^+$ *pin*$^+$) to EJ1352 (*purB pin*) was carried out by the selection of *purB*$^+$. *purB*$^+$ and *pin*$^+$ showed a cotransduction frequency of 38.3% (Table 3). Then, EJ1352 was made *pin*$^+$ by transduction of *pin*$^+$ *zcg-3*::Tn*10* followed by Tet^s selection (20), and the derivative named EJ1419 was used as the recipient for transduction from the *pin* mutants. *purB*$^+$ and the three *pin* alleles were cotransduced at frequencies of 27 to 42% (Table 3). The cotransduction frequencies between *purB*$^+$ and *pin* obtained from the four tests (the last four lines of Table 3) were averaged, and the value 33.3% was used for calculation of the distance between *purB* and *pin*

TABLE 3. Cotransduction between *purB*$^+$ and Tn*10* (Tet^r) and between *purB*$^+$ and *pin*

Donor	Recipient[a]	No. of *purB*$^+$ colonies tested	No. of *purB*$^+$ with unselected donor marker:			Cotransduction (%)
			Tet^r	*pin*$^+$	*pin*	
EJ1396 (*zcg-2*::Tn*10*)	*purB51*	434	121			27.9
EJ1397 (*zcg-3*::Tn*10*)	*purB51*	389	181			46.5
EJ1076 (*pin*$^+$)	*purB51 pin-101*	522		200		38.3
EJ1365 (*pin-8*)	*purB51 pin*$^+$	290			77	26.6
EJ1368 (*pin-16*)	*purB51 pin*$^+$	91			38	41.8
EJ1369 (*pin-20*)	*purB51 pin*$^+$	260			72	27.7

[a] Recipients used were H680 (*purB51*), EJ1352 (*purB51 pin-101*), and EJ1419 (*purB51 pin*$^+$).

by the formula of Wu (28). Figure 1 shows the locus of *pin* relative to *purB* together with the loci *zcg-2*::Tn*10*, *zcg-3*::Tn*10*, and *dadR*, which was based on published data (16). *pin* was inferred to be located at about 26 min on the linkage map (4), flanked by the two Tn*10* insertion sites.

DISCUSSION

Figure 1 was constructed from the cotransduction frequencies from *purB*$^+$ selection only. Therefore, there are some noticeable contradictions when the frequencies from Tet^r selection are taken into account. *zcg-3*::Tn*10* showed a 100% cotransduction frequency with *purB*$^+$ or *pin*$^+$, and *zcg-2*::Tn*10* showed 100% with *purB*$^+$ and 15 to 40% with *pin*. The noteworthy difference between the two selections was that the transduction frequency per PFU was always about 10 times higher for *purB*$^+$ selection. Tn*10* might cause negative interference in recombination with the neighboring region, thus yielding recombinants involving only a larger region. This might partly explain the contradiction described above and the uneven cotransduction frequencies from one test to another as shown in Table 2, but it seems insufficient to explain the frequencies with *zcg-2*::Tn*10* selection. *zcg-2*::Tn*10* showed a higher cotransduction frequency with *purB* than with *pin*, although *purB* is farther from *zcg-2*::Tn*10* than *pin* is on the map as constructed. A nonhomologous region between the donor and recipient chromosomes might be present somewhere in the region from *purB* to *pin*. Anomalous cotransduction data on the region including *purB* have been reported (24).

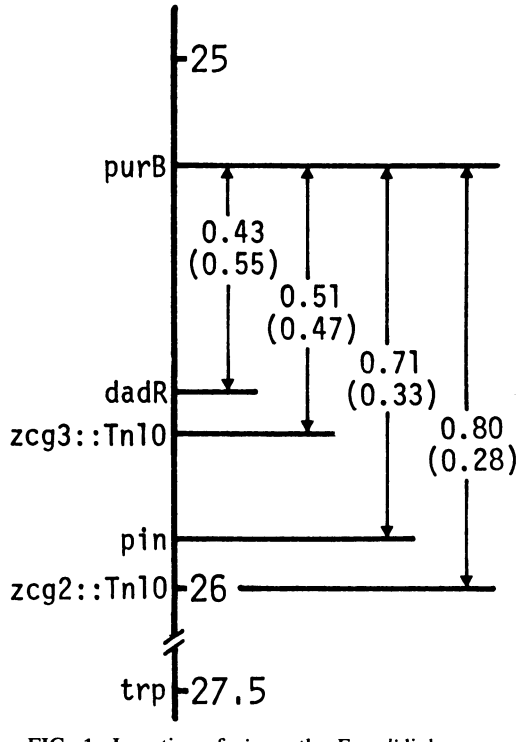

FIG. 1. Location of *pin* on the *E. coli* linkage map (25 to 26 min) (4). The map was constructed from the cotransduction data obtained by *purB*$^+$ selection. Numbers indicate distances from *purB*; numbers in parantheses indicate cotransduction frequencies. Data on *dadR* is from Kuhn and Somerville (16).

The presence or absence of *pin*$^+$ in *E. coli* strains was revealed only when the two *Salmonella H* regions were introduced. The *H1* and *H2* regions have been, so far, introduced to two sorts of *E. coli* strains, and both were found to be *pin*$^+$; one is a derivative of strain W1485 (3, 7), which has been extensively used as one of the ancestral stocks of K-12, and the other is a derivative of strain PA309 (3), which is a descendant of Paris strain P678 and was used in this experiment. Both strains were derived, through different lines, from the wild-type K-12. At first, it was thought that all *E. coli* strains were *pin*$^+$, but in this experiment H680, the strain of P. de Haan, was found to be *pin* deficient. The derivation of H680 could not be traced from the available data. It is still unknown when H680 acquired a *pin* mutation and whether the Pin phenotype was caused by a point mutation or by deletion of the whole gene. The *pin* gene seems to encode a site-specific recombinase that is able to catalyze reversible inversions of the *Salmo-*

nella PD segment and also the C segment of P1 (unpublished data). So far, it is known that three sorts of genes, *Salmonella vh2* (identical with *hin*), P1 *din* (identical with *gin* of Mu?), and *E. coli pin*, give rise to phase variation by causing inversions of the PD segment. The function of pin^+ in *E. coli*, however, is unknown since *E. coli* itself does not show any phenotypic change as a result of *pin* mutations. The rate of phase variation was 10 times higher in the pin^+ din^+ strain (P1 lysogen of EJ1076) than in the pin^+ strain (EJ1076) and much higher in a din^+ background (P1 lysogen of *pin* mutants). These results indicate that the function of P1 din^+ is greater than that of pin^+ and that pin^+ antagonizes din^+ or both genes act against each other.

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