

## Genetic Mapping of the *Salmonella typhimurium pepB* Locus

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Transposon technology has been used to map the *pepB* locus of *Salmonella typhimurium*. This locus is cotransducible by phage P22 with *glyA* and *strB* at min 56 on the *Salmonella* genetic map. The gene order is *strB pepB glyA*.

We have previously reported the identification of six mutations in *Salmonella typhimurium*, each of which affects the production of a different peptidase (5, 7). Except for *pepB*, the map positions of all of these mutations have been determined (6). The *pepB* locus codes for an aminopeptidase (peptidase B) for which no unique substrate has been identified. As a result we cannot conveniently score *pepB* mutations in recombinants from conjugational crosses unless two other peptidase mutations (*pepA* and *pepN*) are present in both the donor and the recipient strains. Thus, considerable strain building would be required to perform the conjugation crosses necessary for determining the approximate map position of the *pepB* locus. The introduction of transposon technology (3) has provided methods for determining map positions of markers that are not easily selected. In this communication we report the application of these methods to the determination of the position of the *pepB* locus on the *Salmonella* genetic map.

The procedure requires the following steps: (i) isolation of a Tn10 insertion near (that is, cotransducible with) the *pepB* locus; (ii) construction of suitable Hfr strains carrying this Tn10 insertion; (iii) crossing these Hfr strains with auxotrophic recipients with selection for prototrophic recombinants and scoring the linkage of the tetracycline resistance phenotype conferred by the Tn10 with the auxotrophic marker. In this procedure the problem of mapping *pepB*, a marker that is difficult to score or select, is replaced by the problem of mapping an insertion conferring tetracycline resistance, an easily selectable and scorable marker. Bacterial strains used in this study are given in Table 1.

To obtain a Tn10 insertion near *pepB*, we isolated a population of strain TN273 [*leu-485 pepN10 pepA1 pepD1 (pepB<sup>+</sup>)*] containing random Tn10 insertions. (Preparation of the Tn10-containing phage P22 vehicle and the procedure used for constructing the population containing random insertions have been described by Kleckner et al. [3, 4].) A generalized transducing lysate prepared on this mixed population was then used to transduce strain TN215 (*leu-485*

*pepN10 pepA1 pepB1 pepD1*) to growth on Leu-Leu as a leucine source. (The only peptidase capable of hydrolyzing Leu-Leu present in the donor strain was peptidase B, so any transductant growing on this peptide should be *PepB<sup>+</sup>*.) Transductants from this cross were replica plated to selective (Leu-Leu) medium containing 25  $\mu$ g of tetracycline per ml. Of about 1,500 Leu-Leu-utilizing transductants tested, three were found to be *Tet<sup>r</sup>* and were purified by two single-colony isolations. P22 lysates of each of these strains were prepared and used to transduce TN215 (*leu-485 pepN10 pepA1 pepB1 pepD1*) to *Tet<sup>r</sup>*. One of the three donor strains gave *Tet<sup>r</sup>* transductants that grew on Leu-Leu. This strain contains a Tn10 insertion about 65% linked to the *pepB* locus. *Tet<sup>r</sup> pepB<sup>+</sup>* (TN846) and *Tet<sup>r</sup> pepB* (TN847) strains were saved from this cross. (The other two *Tet<sup>r</sup>* Leu-Leu-utilizing transductants did not contain a Tn10 insertion near *pepB* and presumably arose by double transduction events.) The presence of peptidase B (and the absence of peptidases A, N, and D) in TN846 was confirmed by staining for peptidase activity after gel electrophoresis of a crude extract (7).

Hfr strains containing the Tn10 insertion near *pepB* were constructed by selecting *Tet<sup>r</sup>* in a transduction cross with TN847 as donor and SA534 and SA966 (11) as recipients. The resulting Hfr strains TN844 and TN845 were shown by appropriate transduction crosses to have inherited the *pepB* allele from their donor parent.

These Hfr strains were each crossed with several different auxotrophs, using the mating procedure described by Sanderson et al. (11). Prototrophic recombinants were selected, and these recombinants were scored for inheritance of *Tet<sup>r</sup>* by replica plating to nutrient agar containing 25  $\mu$ g of tetracycline per ml. Of 10 auxotrophic markers used as recipients, *Tet<sup>r</sup>* was more highly linked to *purG* and *glyA* (80 to 95% linkage) than to any other markers tested (linkage, 50% or less). These results strongly suggested Tn10 insertion (and *pepB*) maps very close to min 56 on the *Salmonella* map (10).

To confirm this suggestion and to define the

precise map position of *pepB*, P22 transduction crosses were carried out with strain TN847 (Tn10 near *pepB*) as donor and a strain carrying *glyA1* as recipient. The results of this cross are shown in Table 2, line 1. These results show that the Tn10 insertion near *pepB* is about 10% cotransducible with *glyA*. The 10 Tet<sup>r</sup> Gly<sup>+</sup> transductants from this cross were purified and used as donors in transduction crosses with TN856 [*leu-485 pepN10 pepA1 pepD3 (pepB<sup>+</sup>)*] with selection for Tet<sup>r</sup> recombinants. All 10 strains gave recombinants that failed to grow on Leu-Leu. (On the average, 64% of the Tet<sup>r</sup> transductants did not grow on Leu-Leu; i.e., they were *pepB*.) This result shows that each of the 10 Tet<sup>r</sup>

Gly<sup>+</sup> transductants (from the cross shown in Table 2, line 1) had received the donor *pepB* allele and therefore that the *pepB* locus itself is also cotransducible with *glyA*. (The Tet<sup>r</sup> Gly<sup>+</sup> transductants could not be scored directly for *pepB* because the recipient strain is *pepN<sup>+</sup> pepA<sup>+</sup>* and grows on Leu-Leu regardless of the *pepB* genotype.)

Several other crosses were carried out to confirm these results. The Tn10 insertion near *pepB* is also linked to *glyA* when Gly<sup>+</sup> (rather than Tet<sup>r</sup>) recombinants are selected (Table 2, line 2), although the cotransduction frequency is lower when Gly<sup>+</sup> is the selected marker. Linkage between a Tn10 insertion in *glyA* and *pepB* is

TABLE 1. Bacterial strains<sup>a</sup>

Strain	Genotype	Origin
TN217	<i>leu-485 pepN10 pepA1 pepD1 pepB3</i>	Isolated as described in reference 7
TN218	<i>leu-485 pepN10 pepA1 pepD1 pepB4</i>	Isolated as described in reference 7
TN273	<i>leu-485 pepN10 pepA1 pepD1 (pepB<sup>+</sup>)</i>	7
TN360	<i>leu-485 pepN10 pepA1 pepD3 (pepB<sup>+</sup>)</i>	Isolated as described in reference 7
TN822	<i>leu-485 pepN10 pepA1 pepB1 pepD3</i>	Miller and Green (manuscript in preparation)
TN844	<i>serA12 zfg801::Tn10 pepB1 HfrK4</i>	TN847 × SA534 (11)
TN845	<i>leuBCD39 ara-7 zfg801::Tn10 pepB1 HfrK19</i>	TN847 × SA966 (11)
TN846	<i>leu-485 pepN10 pepA1 pepD1 zfg801::Tn10 (pepB<sup>+</sup>)</i>	<i>zfg801::Tn10</i> × TN215 (see text)
TN847	<i>leu-485 pepN10 pepA1 pepD1 zfg801::Tn10 pepB1</i>	<i>zfg801::Tn10</i> × TN215 (see text)
TN856	<i>leu-485 pepN10 pepA1 pepD3</i>	Miller and Green (manuscript in preparation)
TN880	<i>leu-485 pepN10 pepA1 pepB1 pepD3 glyA540::Tn10</i>	TT418 × TN822 (see text)
TN926	<i>leu-485 pepN10 pepA1 pepB1 pepD3 strB1201</i>	Spontaneous <i>strB</i> from TN822 <sup>b</sup>
TT315	<i>purG1739::Tn10</i>	J. R. Roth
TT418	<i>glyA540::Tn10</i>	J. R. Roth
SA534	<i>serA13 rfa-3058 HfrK4</i>	11
SA966	<i>leuBCD39 ara-7 HfrK19</i>	11

<sup>a</sup> All strains are derived from *S. typhimurium* LT2. The nomenclature for Tn10 insertions near *pepB* follows the proposal of Hong and Ames (2) as modified by Chumley et al. (1).

<sup>b</sup> Spontaneous mutations at *strB* conferring low-level resistance to streptomycin and a requirement for thiamine and nicotinic acid were selected as described by Wyche et al. (12).

TABLE 2. P22 transduction crosses<sup>a</sup>

Donor	Recipient	Selected marker	Unselected marker	% Linkage (no. of transductants/total)
1. TN847 (Tn10 near <i>pepB1</i> )	<i>glyA1</i>	Tet <sup>r</sup>	Gly <sup>+</sup>	10 (10/100)
2. TN847	<i>glyA1</i>	Gly <sup>+</sup>	Tet <sup>r</sup>	1 (1/100)
3. TT418 ( <i>glyA::Tn10</i> )	TN822	Tet <sup>r</sup>	PepB <sup>+</sup> <sup>b</sup>	3 (3/100)
4. TN360 ( <i>pepB<sup>+</sup></i> )	TN880	PepB <sup>+</sup> <sup>b</sup>	Tet <sup>r</sup>	15 (4/26)
5. TT315 ( <i>purG::Tn10</i> )	TN822	Tet <sup>r</sup>	PepB <sup>+</sup> <sup>b</sup>	<1 (0/100)
6. TT315 ( <i>purG::Tn10</i> )	<i>glyA1</i>	Tet <sup>r</sup>	Gly <sup>+</sup>	26 (21/80)
7. TN846 (Tn10 near <i>pepB<sup>+</sup></i> )	TN926	StrB <sup>+</sup> <sup>c</sup>	PepB <sup>+</sup> <sup>b</sup>	44 (44/100) <sup>d</sup>
8. TT418 ( <i>glyA::Tn10</i> )	TN217	Tet <sup>r</sup>	PepB <sup>+</sup> <sup>b</sup>	1.2 (7/570)
9. TT418	TN218	Tet <sup>r</sup>	PepB <sup>+</sup> <sup>b</sup>	3.5 (3/86)

<sup>a</sup> Transduction was carried out as described by Roth (8), using P22 HT 12/4 *int3*. Media and growth conditions are given in reference 7. Full genotypes are given in Table 1.

<sup>b</sup> Recombinants carrying the *pepB<sup>+</sup>* allele were scored or selected for growth on Leu-Leu as a leucine source.

<sup>c</sup> Selection for growth in the absence of thiamine and nicotinic acid (12).

<sup>d</sup> The frequency of recombinant classes from this cross (PepB<sup>+</sup> Tet<sup>r</sup>, 12/100; PepB<sup>-</sup> Tet<sup>r</sup>, 1/100) suggests that *zfg801::Tn10* is located between *pepB* and *glyA*.

demonstrated by the crosses shown in Table 2, lines 3 and 4.

To orient the *pepB* locus with respect to other markers linked to *glyA* (9), we carried out the cross shown in Table 2, line 5. This cross shows that *pepB* is unlinked (<1%) to a Tn10 insertion in *purG*. Since the Tn10 insertion in *purG* is 23% linked to *glyA* (Table 2, line 6), *pepB* must lie on the opposite side of *glyA* from *purG*. If this is correct, *pepB* should be linked to *strB* (9). (The *strB* locus is incorrectly placed on the current *Salmonella* map [10].) The cross shown in Table 2, line 7, shows that *strB* and *pepB* are indeed cotransducible and establishes the order *strB pepB purG*. The linkage relationships between the markers in this region are summarized in Fig. 1. The crosses shown in Table 2, lines 8 and 9, show that two other *pepB* alleles (*pepB3* and *pepB4*) are also cotransducible with *glyA*.

The *pepB* gene is the sixth *pep* locus to be mapped. None of these six loci is located near any other (6). Since we now know the map positions of all six loci, we can use transposon technology to construct an isogenic series of strains carrying various combinations of *pep* alleles. Although the procedures used for determining the *pepB* map position involve a number

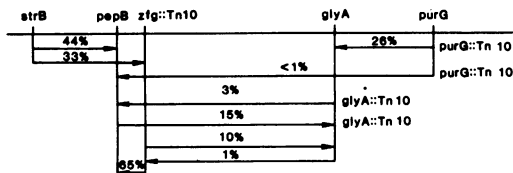


FIG. 1. Genetic map of the *pepB* region of the *Salmonella* chromosome. Linkage data are for phage P22 transduction crosses. The arrow points to the unselected marker.

of steps, each step is straightforward and the products can be easily checked. We have found that the use of these transposon methods greatly simplifies difficult mapping problems.

This work was supported by Public Health Service research grant AI10333 from the National Institute of Allergy and Infectious Diseases.

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