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Genetic Mapping of *Salmonella typhimurium* Peptidase Mutations

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The map positions of three loci, each specifying a different peptidase, have been determined in *Salmonella typhimurium*. Mutations in *pepN* (leading to loss of peptidase N [1974]) are co-transducible with *pyrD*. The order of markers in this region is *put pyrD pepN*. Mutations in *pepA* (leading to loss of peptidase A [1974]) are co-transducible with *pyrB* and *argI*. The relative orientation of these markers is *pepA argI pyrB*. Mutations in *pepD* (leading to loss of dipeptidase, peptidase D) are co-transducible with *proBA* and *gxu*. The order of these markers is *pepD gxu pro*.

Intracellular peptidases in bacteria play roles in several important physiological processes. They function in the catabolism of exogenously supplied peptides (7, 12, 13), are necessary for the degradation of intracellular protein to free amino acids observed during starvation for a carbon source (8; C. Yen, unpublished observations), and must perform N-terminal modifications to convert precursor proteins to their mature forms (8). The isolation of mutant strains of *Salmonella typhimurium* that are deficient in several distinct broad-specificity peptidase activities has recently been reported (5, 7). This paper describes the genetic mapping of three classes of these mutations.

MATERIALS AND METHODS

Bacterial strains. The multiply marked bacterial strains used in this work are described in Table 1. All are derived from *S. typhimurium* LT2, except strains carrying the *proAB47* marker. These strains are LT7 derivations.

Media and growth conditions. Media and growth conditions have been described previously (7).

Conjugation. Matings between Hfr and F^- strains were performed by plating 0.1 ml each of both donor and recipient (overnight cultures grown in nutrient broth) on appropriately supplemented minimal glucose medium.

Transduction. Transduction with P22 *int-4* was performed as described by Roth (9).

Preparation of crude cell extracts. Crude cell extracts were made by disrupting stationary-phase cells in a French pressure cell as described previously (7).

Electrophoresis and peptidase activity stain. Electrophoresis was performed in polyacrylamide slab gels (apparatus manufactured by Hoefer Scientific, San Francisco). The composition of the gels and the peptidase activity stain have been described previously (7).

RESULTS

Map position of pepN. Mutations in *pepN* lead to loss of a broad-specificity aminopeptidase (peptidase N) capable of hydrolyzing amino acid β-naphthylamides (7). Such mutants have been isolated by screening for colonies that do not hydrolyze the chromogenic peptidase N substrate L-alanine β-naphthylamide. To determine the approximate map position of *pepN*, Hfr strains carrying $pepN^-$ mutations (TN26, TN28, Table 1) were crossed with a series of auxotrophic $pepN^+$ strains, protrotrophic recombinants were selected, and the frequency of the donor $pepN^-$ allele among these recombinants was determined. The results of these crosses showed that *pepN* is significantly linked to markers that lie between 40 and 60 min on the *Salmonella* map. In a cross between TN26 (Hfr K9 [11]) and *pyrD13*, about 95% of the Pyr^+ recombinants received the donor $pepN^-$ allele. This high linkage suggests that *pepN* and *pyrD* might be jointly transduced by phage P22. The data presented in Table 2 show that all of the *pepN* mutations tested are indeed jointly transduced with *pyrD* at high frequency (72 to 90%).

The order *pyrC put pyrD* has been established previously (10) by the following observations: (i) *pyrC* and *pyrD* are not jointly transduced; (ii) *pyrC* and *put* are jointly transduced by P22, but *put* point mutations are not jointly transduced with *pyrD* (B. Ratzkin and J. Roth, personal communication); and (iii) two *put* deletions (*put-521* and *put-544*) are each jointly transduced with both *pyrC* and *pyrD* (B. Ratzkin and J. Roth, personal communication). To establish the orientation of *pepN* with respect to these markers, the crosses depicted in Table 3

TABLE 1. *Bacterial strains*

Strain	Genotype
TN2	*leu-485 pepN10*
TN5	*leu-485 pepN13*
TN25	*proAB47 leu-1551 pepN23*
TN26	*thr-9 pepN24* HfrK9
TN28	*serA13 pepN26* HfrK6
TN50	*proAB47 leu-1552 pepN27*
TN52	*proAB47 leu-1552 pepN29*
TN102	*leu-485 pepN10 pepA1*
TN213	*leu-485 pepN10 pepA1 pepD1*
TN215	*leu-485 pepN10 pepA1 pepD1 pepB1*
TN228	*leu-485 pepN10 pepA2*
TN235	*leu-485 pepN10 pepA1 proB25 gxu-5*
TN298	*leu-485 pepN10 pepA3*
TN299	*leu-485 pepN10 pepA4*
TN300	*leu-485 pepN10 pepA5*
TN302	*leu-485 pepN10 pepA7*
TN303	*leu-485 pepN10 pepA8*
TN304	*leu-485 pepN10 pepA9*
TN305	*leu-485 pepN10 pepA10*
TN320	*ara-7 leu-39 his-6857 pepN64 pepA11* HfrK16
TN372	*pyrD13 pepN76*
TR2140	*putB609*
TR2287	*proB25 gxu-5*
TR2962	*leuD798 fol-101 pyrB64 argI547*

TABLE 2. *Joint transduction of pepN with pyrD*

Phage donor	Recipient	Selected marker	Unselected marker	No. tested	% Carrying unselected marker
TN2 (*pepN10*)	*pyrD13*[a]	Pyr$^+$	PepN$^-$	100	90
TN5 (*pepN13*)	*pyrD13*	Pyr$^+$	PepN$^-$	100	94
TN26 (*pepN24*)	*pyrD13*	Pyr$^+$	PepN$^-$	100	72
TN28 (*pepN26*)	*pyrD13*	Pyr$^+$	PepN$^-$	100	87
TN50 (*pepN27*)	*pyrD13*	Pyr$^+$	PepN$^-$	100	87
TN52 (*pepN29*)	*pyrD13*	Pyr$^+$	PepN$^-$	100	86

[a] Growth properties of the strain (growth on orotic acid and uracil but not on dihydroorotic acid) confirm that it carries a *pyrD* mutation (15).

were performed. The results of these crosses show: (i) the two *put* deletions (*put-521* and *put-544*) are jointly transduced with both *pyrD* and *pepN* whereas a *put* point mutation (*putB609* [TR2140]) is not jointly transduced with either *pyrD* or *pepN;* (ii) the most likely order is order 1 (*put pyrD pepN*) (Table 3). If order 2 (*put pepN pyrD*) were correct, the frequency of inheritance of the donor *put*$^-$ allele should be lower among recombinants that receive the recipient *pepN*$^-$ allele than among recombinants receiving *pepN*$^+$. The data in Table 3 show that just the opposite is true; the frequency of inheritance of *put*$^-$ is higher among the *pepN*$^-$ recombinants than among the *pepN*$^+$ class.

Map position of pepA. Mutations in *pepA* (7) lead to loss of a broad-specificity aminopeptidase similar to the *Escherichia coli* peptidase purified and characterized by Vogt (14). *pepA* mutants have been isolated in a *leu*$^-$ *pepN*$^-$ strain by penicillin selection for failure to use Leu-Ala-NH_2 as a leucine source. In addition, *pepA* mutants fail to use L-leucinamide as a nitrogen source.

Derivatives of *leu*$^-$ *pepN*$^-$ *pepA*$^-$ strain carrying various other markers were constructed and used as recipients in crosses with several Hfr strains. Recombinants from these crosses were scored for the presence of the donor *pepA*$^+$ allele. Results of these crosses suggested that *pepA* lies in the region between zero and about 20 min on the *Salmonella* map. Various markers in this region were tested for joint transduction with *pepA*. Results of transductional crosses (Table 4) show that *pepA1* is jointly transduced with both *pyrB* and *argI* located at zero min on the map. In addition, the crosses described in Table 4 constitute reciprocal three-point tests. The results presented in Table 4 line 1 indicate that when PepA$^+$ recombinants are selected in a cross between an *argI*$^-$ *pyrB*$^-$ donor and a *pepA*$^-$ recipient, PepA$^+$ Arg$^+$ Pyr$^-$ recombinants are rare (0/82). This is expected if order 1 (*pepA argI pyrB*) is correct. Recombinants of this type do arise in the reciprocal cross, however. If the *argI*$^-$ *pyrB*$^-$ strain is the recipient, the *pepA*$^-$ strain is the donor (Table 4, line 2), and Arg$^+$ recombinants are selected, PepA$^+$

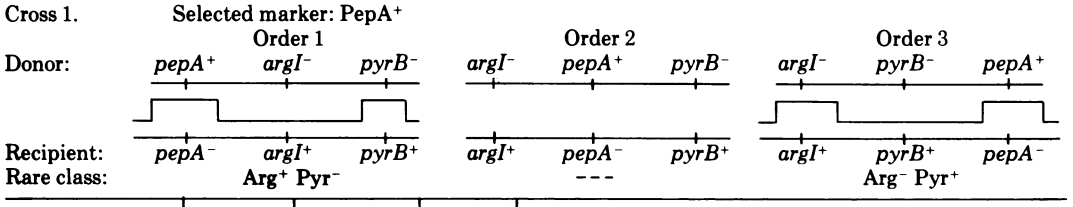
TABLE 3. *Mapping of pepN by transduction*

	Order 1			Order 2		
Donor:	*put*$^-$	*pyrD*$^+$	*pepN*$^+$	*put*$^-$	*pepN*$^+$	*pyrD*$^+$
Recipient: (TN372)	*put*$^+$	*pyrD*$^-$	*pepN*$^-$	*put*$^+$	*pepN*$^-$	*pyrD*$^-$
Selected marker:	Pyr$^+$					

Donor	No. tested	Selected class	Unselected class	No.	Frequency
put-521	581	PyrD$^+$ PepN$^+$	Put$^+$	526	—
			Put$^-$	14	14/540 (2.6%)
		PyrD$^+$ PepN$^-$	Put$^+$	38	—
			Put$^-$	3	3/41 (7.3%)
put-544	796	PyrD$^+$ PepN$^+$	Put$^+$	736	—
			Put$^-$	13	13/749 (1.7%)
		PyrD$^+$ PepN$^-$	Put$^+$	43	—
			Put$^-$	4	4/47 (8.5%)
TR2140	520	PyrD$^+$ PepN$^+$	Put$^+$	490	—
			Put$^-$	0	—
		PyrD$^+$ PepN$^-$	Put$^+$	30	—
			Put$^-$	0	—

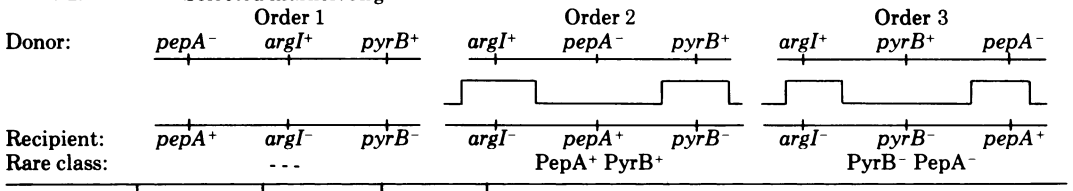
TABLE 4. *Mapping of pepA by transduction*

Cross 1. Selected marker: PepA⁺



Donor	Recipient	Selected marker	No. tested	Recombinant classes					
				Arg	Pyr	No.	%	Arg⁻ total	Pyr⁻ total
TR2962 (*argI⁻ pyrB⁻*)	TN102 (*pepA1*)	PepA⁺	82	+	+	28	34	66%	34%
				+	−	0	0		
				−	+	19	23		
				−	−	35	43		

Cross 2. Selected marker: Arg⁺



Donor	Recipient	Selected marker	No. tested	Recombinant classes					
				PepA	Pyr	No.	%	PepA⁻ total	Pyr⁺ total
TN102	TR2962	Arg⁺	200	+	+	46	23	63%	42%
				+	−	28	14		
				−	+	38	19		
				−	−	87	44		

TABLE 5. *Joint transduction of pepA with pyrB and argI*

Donor	Recipient	Selected marker	No. tested	Frequency of recombinant class (%)					
				Arg⁺ Pyr⁺	Arg⁻ Pyr⁻	Arg⁺ Pyr⁻	Arg⁻ Pry⁺	Arg⁻ total	Pyr⁻ total
TR2962	TN228 (*pepA2*)	PepA⁺	80	46	34	1	20	54	35
TR2962	TN298 (*pepA3*)	PepA⁺	78	46	36	0	18	54	36
TR2962	TN299 (*pepA4*)	PepA⁺	81	36	33	0	31	64	33
TR2962	TN300 (*pepA5*)	PepA⁺	63	48	25	0	37	62	25
TR2962	TN302 (*pepA7*)	PepA⁺	64	41	34	0	25	59	34
TR2962	TN303 (*pepA8*)	PepA⁺	83	24	46	0	30	76	46
TR2962	TN304 (*pepA9*)	PepA⁺	79	43	42	0	15	57	42
TR2962	TN305 (*pepA10*)	PepA⁺	69	42	38	0	20	58	38
TR2962	TN320 (*pepA11*)	PepA⁺	75	39	35	0	26	61	35

Arg⁺ Pyr⁻ recombinants are found. The higher frequency of joint transduction between *pepA* and *argI* (66%) than between *pepA* and *pyrB* (35%) also is consistent with the order *pepA argI pyrB*. Table 5 presents data indicating that all of the *pepA* alleles we have obtained are jointly transduced with *argI* and *pyrB*. These data also confirm the order *pepA argI pyrB* since: (i) the *pepA* alleles tested are 54 to 76% co-transducible with *argI* but only 25 to 46% co-transducible with *pyrB*, and (ii) the Arg⁺ Pyr⁻ class is rare.

Map position of pepD. Mutations in *pepD* lead to loss of a dipeptidase and have been obtained from a *leu⁻ pepN⁻ pepA⁻* strain by penicillin selection for mutants unable to use Leu-Gly as a leucine source (7). By chance it was observed that a large deletion mutation *proAB47* (3) leads to loss of the dipeptidase activity (G. L. McHugh, M.S. thesis, Case

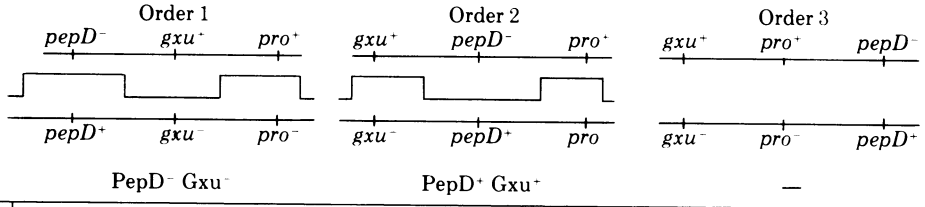
Western Reserve Univ., Cleveland, Ohio, 1972). Pro$^+$ transductants were isolated from a cross using phage grown on LT2 (wild type) as a donor and *proAB47* as recipient. All of the transductants tested (4/4) regained the dipeptidase activity as well as a functional *pro* region. These results suggested that *pepD* probably lies near the *proBA* locus. The *proBA* locus is also co-transducible with *gxu*, and the order of these markers is known to be (reading clockwise on the Sanderson map) *gxu proBA* (10). The *gxu* locus specifies the enzyme hypoxanthine-guanine phosphoribosyltransferase (1). Mutants at this locus are resistant to 8-azaguanine. Mutations in the *proBA* locus lead to a requirement for proline. To determine the orientation of *pepD* with respect to these markers, the crosses described in Table 6 were performed. The data in Table 6 demonstrate that *pepD1* (a *pepD* point mutation) is jointly transduced with *proBA* and with *gxu*. In cross 1 (Table 6) the frequency of joint transduction between *pro* and *pepD* is about 65%, whereas *pro* and *gxu* are jointly transduced at a frequency of about 73%. In cross 2, 50% of the *pepD*$^+$ recombinants also receive the donor *pro*$^-$ marker and 77% receive *gxu*$^-$ from the donor. The data from these crosses strongly suggest the order *pepD gxu proAB* since in both crosses the rare class is that expected from this order.

Itikawa and Demerec have isolated a series of *pro* deletion mutations some of which extend out of the *pro* region to the right, i.e., toward *ataA*, the P22 attachment site, others to the left (toward *gxu*), whereas still others are large deletions in which the *pro* locus and regions on either side have been lost (3). Another series of deletion mutations affecting this region of the chromosome has been isolated by Kemper ([4] and personal communication). One end point of all these deletions lies in or near *supQ* (see Table 7). Some of these deletions extend into the *proBA* region, others extend beyond *proB* but still retain a functional *gxu* locus, still others extend into or through *gxu* (1; J. Kemper, personal communication). If the order *pepD gxu pro* is correct, some of these deletion mutatant strains may be *pepD*$^+$ *gxu*$^-$ *pro*$^-$ or *pepD*$^-$ *gxu*$^-$ *pro*$^-$, but no *pepD*$^-$ *gxu*$^+$ *pro*$^-$ strains should be found.

These predictions were tested in either of two ways. Extracts of some of the deletion mutant strains were prepared and electrophoresed on polyacrylamide, and the presence or absence of

TABLE 6. *Mapping of pepD by three-point crosses*

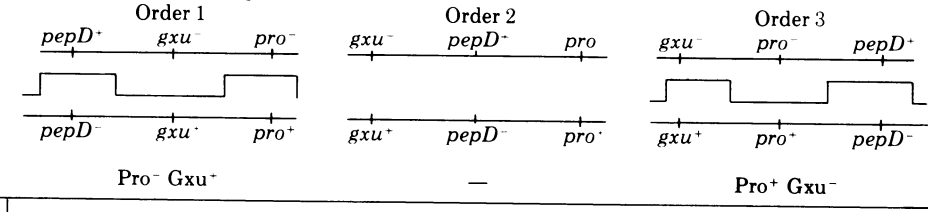
Cross 1. Selected marker: Pro$^+$



Rare class: PepD$^-$ Gxu$^-$ PepD$^+$ Gxu$^+$ —

No. tested	Frequency of recombinant class (%)					
	PepD$^-$ Gxu$^+$	PepD$^-$ Gxu$^-$	PepD$^+$ Gxu$^+$	PepD$^+$ Gxu$^-$	PepD$^+$ total	Gxu$^-$ total
95	64	1	9	25	65	73

Cross 2. Selected marker: PepD$^+$

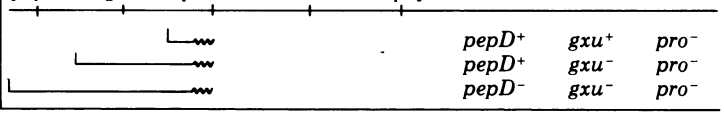


Rare class: Pro$^-$ Gxu$^+$ — Pro$^+$ Gxu$^-$

No. tested	Frequency of recombinant class (%)					
	Pro$^-$ Gxu$^-$	Pro$^-$ Gxu$^+$	Pro$^+$ Gxu$^-$	Pro$^+$ Gxu$^+$	Pro$^-$ total	Gxu$^-$ total
64	50	0	27	23	50	77

TABLE 7. *Deletion mapping of pepD*[a]

Proposed genotype			Mutant
pepD	*gxu*[b]	*pro*	
+	+	−	*proB25*,[c] *proA107*,[c] *supQ262*, *284*,[c] *285*,[c] *308*, *309*, *310*, *320*, *321*, *326*, *327* *328*, *332*, *335*, *336*, *340*, *341*, *347*, *364*, *381*, *382*, *383*, *385*
+	−	−	*supQ307*,[c] *321*, *318*, *319*, *337*, *348*, *357*, *359*, *361*, *371*, *375*, *384*
−	−	−	*proAB47*,[c] *53*,[c] *126*,[c] *supQ274*, *302*,[c] *305*,[c] *306*,[c] *311*, *313*, *356*



[a] The presence or absence of *pepD* was determined genetically by using phage grown on the strain to be tested in a cross with TN215 (*leu-485 pepN10 pepA1 pepB1 pepD1*) as recipient. Leu-Gly-utilizing recombinants were selected and scored for their ability to hydrolyze L-alanyl-β-naphthylamide and to utilize Leu-Gly-Gly as a leucine source. Transductants that failed to hydrolyze ANA and also failed to utilize Leu-Gly-Gly but grew on Leu-Gly were scored as PepD+ (7). For several of the crosses, the PepD+ recombinants were tested for the presence of the donor deletion. A large fraction of the PepD+ recombinants from these crosses were Pro−. None of the other types of recombinants (*pepN*+ or *pepA*+; cf. [7]) were Pro−

[b] *gxu*−, Resistance to 8-azaguanine. Resistance to 8-azaguanine was tested by placing a few crystals on an appropriately supplemented minimal-glucose plate and noting the presence or absence of a zone of growth inhibition after overnight incubation. These data confirm those previously reported by Gots et al. (1) (*pro* mutants) and by Kemper (personal communication [*supQ* mutants]).

[c] Presence or absence of peptidase D activity confirmed by gel electrophoresis of a crude extract (7).

the band of peptidase D activity was determined (7). For other strains, phage lysates grown on the deletion mutant were used in transductional crosses with TN215 (*leu-485 pepN10 pepA1 pepD1 pepB1*) as recipient. Recombinants were selected for growth on Leu-Gly and screened to determine which ones were PepD+ (7) (Table 7). As expected if the correct order is *pepD gxu pro*, none of these deletion mutant strains is *pepD*− *gxu*+. Some of the *supQ* deletions have an end point between *pepD* and *gxu* whereas others extend through *pepD*. The left end points of these deletions are shown on the map in Table 7.

Since several of the deletion mutations extend through *pro gxu pepD*, it is possible to introduce a stable *pepD* mutation into any azaguanine-sensitive strain. Phage grown on such a deletion mutant can be used as donor in a cross-selecting 8-azaguanine resistance on a plate containing both 8-azaguanine and proline. Although many azaguanine-resistant mutants appear on the control plates (in the absence of phage), the transductant colonies will be resistant because they have received the deletion mutation and can be identified because they are Pro−. These transductants are also PepD−. Such transductions have been carried out using *proAB47* as donor.

P22 specialized transducing phages carrying the *proAB* region of *E. coli* have been isolated by Hoppe and Roth (2). The presence of *pepD* on several of these phages has been shown by detecting the peptidase D band in crude extracts of Pro+ transductants derived from crosses using the specialized transducing phage lysate as donor and *proAB47* as recipient. Therefore, *pepD* seems to be located near *proBA* in *E. coli* as well as in *Salmonella*.

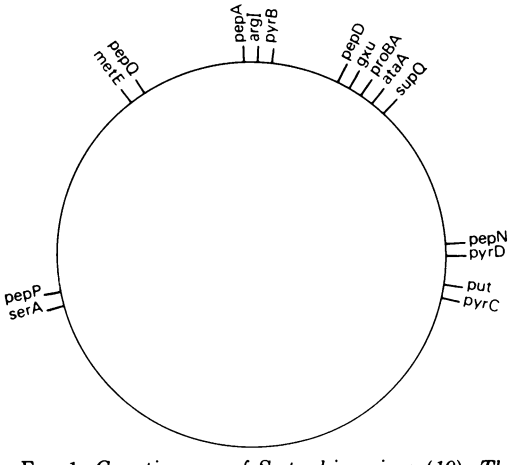


FIG. 1. *Genetic map of S. typhimurium (10). The map positions of three types of mutations described in this paper, as well as two other types of peptidase mutations previously described (5), are shown. The markers pepP and serA are co-transducible, but their relative orientation is not known.*

DISCUSSION

Figure 1 is a genetic map of *S. typhimurium* showing the locations of all the known loci at which mutations leading to loss of a peptidase occur. Clearly no clustering of peptidase genes is observed. If there is any joint regulation of these peptidases, this regulation is not based on a peptidase operon. Also, none of the peptidases are located near any other known genes to which they might be reasonably imagined to be functionally related.

Location of each of these peptidase genes near well-known nutritional markers allows the construction by transduction of strains containing various combinations of peptidase alleles.

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