JOURNAL OF BACTERIOLOGY, Oct. 1972, p. 17-29
Vol. 112, No. 1

Printed in U.S.A.

Deletion Mapping of the Genes Coding for HPr and Enzyme I of the Phosphoenolpyruvate: Sugar Phosphotransferase System in *Salmonella typhimurium*

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Received for publication 21 June 1972

Sugars transported by a bacterial phosphoenolpyruvate:sugar phosphotransferase system (PTS) require two soluble proteins: HPr, a low-molecular-weight phosphate-carrier protein, and enzyme I. The structural genes coding for HPr (*ptsH*) and Enzyme I (*ptsI*) are shown to be cotransducible in *Salmonella typhimurium*. The gene order of this region of the *Salmonella* chromosome is *cysA-trzA-ptsH-ptsI...(crr)*. A method for the isolation of *trzA-pts* deletions is described. One class of *pts* deletions extends through *ptsH* and into *ptsI;* a second class includes both *ptsH* and *ptsI* and extends into or through the *crr* gene. The *crr* gene either codes for or regulates the synthesis of a third PTS protein (factor III) which is sugar-specific. A hypothesis is presented for a mechanism of deletion formation.

A bacterial phosphotransferase system (PTS) catalyzes the transfer of phosphate from phosphoenolpyruvate (PEP) to a variety of sugars (7-9, 13). The PTS has been implicated both in the transport and accumulation of various carbohydrates across the plasma membrane (13, 18) and in the regulation of synthesis of certain inducible enzyme systems (14). In all cases thus far examined in detail, the net transfer of phosphate from PEP to a given sugar requires four proteins. Two PTS proteins, HPr and enzyme I, are required to phosphorylate all sugars transported by the PTS. In addition, phosphorylation of a particular PTS sugar requires two sugar-specific proteins, at least one of which is a constituent of the cell membrane. Most of the sugar-specific protein pairs are inducible. The membrane-bound, sugar-specific proteins are designated enzymes II-A and II-B, and the cytoplasmic, sugar-specific proteins are called factor III. The enzymological role of these proteins in the overall sugar phosphorylation reaction has been described in detail (1, 8, 9, 13). This process involves the sequential transfer of a phosphoryl group from PEP to enzyme I, to HPr, to one of the sugar-specific proteins (either enzyme II-A or factor III), and finally to the sugar. The last step requires a membrane-bound enzyme II-B. The proteins isolated from *Salmonella typhimurium* and from *Escherichia coli* appear to be identical in all properties compared thus far; e.g., the HPr proteins from both gram-negative organisms (1) exhibit similar amino acid compositions and molecular weights (9,600).

The most definitive information available concerning the physiological functions of the PTS has been derived from the study of mutants defective in either HPr or enzyme I (13-15, 18). In our experience, some enzyme I point mutants are leaky and all HPr point mutants tested are leaky and unstable; the isolation and characterization of *pts* deletions therefore should provide material for an unequivocal determination of the physiological role(s) of the PTS. Although preliminary evidence has been presented that both in *Salmonella* and in *Escherichia* the structural genes coding for HPr and enzyme I may constitute an operon (6, 15), no deletions of either or both of these genes have been unequivocally characterized.

In *Salmonella*, the structural genes coding

for HPr (*ptsH*) and enzyme I (*ptsI*) were reported to be non-cotrasducible by bacteriophage P22; *ptsH* (previously designated *carB*) was localized between *cysA* and *his* (*purC-cysA-carB...[his]*) such that *cysA* and *carB* were non-cotransducible, and *ptsI* (previously designated *carA*) was mapped between *azi* and *pro* (11). In *Escherichia*, mating and transduction experiments indicated that *ptsH* and *ptsI* are adjacent, and a single reciprocal three-point test suggested that these genes are in the following order: (*purC*)...*ptsI-ptsH-supN* (6). We demonstrate here that these two *pts* genes in *Salmonella* are cotransducible with each other and with *cysA* and *trzA*, the latter a gene coding for sensitivity to 1,2,4-triazole (16). The *Salmonella* gene order most strongly favored by reciprocal three-point tests and deletion mapping is *cysA-trzA-ptsH-ptsI...(crr)*. The *crr* gene either codes for or regulates the synthesis of a constitutive factor III. (In addition to the enzyme II-A:enzyme II-B system described above which can catalyze the transfer of the phosphoryl group from phospho-HPr to glucose [or methyl α-glucoside], mannose, and fructose, there exists an additional system which is composed of a membrane-bound enzyme II-B′ and a constitutive, soluble factor III which can be assayed with either methyl α-glucoside or thiomethyl β-D-galactopyranoside [TMG; W. Kundig and S. Roseman, *in preparation*].) A selection procedure was devised to isolate deletions of these *pts* loci. The recovery of such multisite mutations demonstrates that the complete absence of either HPr and enzyme I or HPr, enzyme I, and factor III is not lethal to cells. In addition, a strain shown genetically to contain an episome covering *ptsH* and *ptsI* (J. H. Wyche et al., Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 43, 1972) produces several-fold the amount of HPr and enzyme I present in its haploid parents.

MATERIALS AND METHODS

Media. Difco nutrient broth served as complex medium. Medium A (5), modified and used as chemically defined minimal medium, contained the following components per liter of distilled water: $(NH_4)_2SO_4$, 1 g; K_2HPO_4, 10.5 g; KH_2PO_4, 4.5 g; $MgSO_4$, 0.1 g; and granulated agar (BBL), 15 g. The agar was omitted when liquid medium was used for growth of cell cultures. Carbon sources were used at final concentrations of 0.2%; lactate was added to the medium prior to autoclaving, whereas sugars were added to the hot medium immediately after autoclaving because prior sterilization of the sugars was found unnecessary (routine inspection of uninoculated plates revealed no contaminants after prolonged incubation at 37 C). When required, supplements of amino acids, purines, and pyrimidines were added at final concentrations of 20 μg/ml each. Levine's EMB without lactose (BBL) supplemented with the appropriate sugar at 1% was used as the indicator medium.

Bacterial strains. Table 1 lists the strains and their derivatives used in this study. Table 2 describes the pertinent phenotypic characteristics of representative strains. Point mutations in *ptsH* or *ptsI* were isolated by standard penicillin enrichment techniques after mutagenesis and growth in medium A containing 0.2% lactate plus the required supplements. Point mutations in *trzA* were induced and selected for on medium A agar plates containing 0.2% lactate plus 10 mM 1,2,4-triazole (Sigma Chemical Co.) and any required supplements. Either a sterile filter-paper disc saturated with diethylsulfate or crystals of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine was used as mutagen. Reversion studies also were performed by this technique. Deletions of the *trzA-ptsHI* and *trzA-ptsHI-crr* types were isolated as described in Results under "Deletions encompassing pts loci."

Transduction experiments. The preparation and storage of P22 transducing lysates and the performance of transduction crosses have been described (2); P22 mutant L-7 (19) was used for the three-point tests, and KB1 phage (3) was used in deletion mapping. In three-point tests involving *trzA* or *ptsH* point mutations, or both, resistance to 1,2,4-triazole and the HPr^- (*ptsH*) phenotype were unselected. Recombinant clones were suspended in sterile 0.9% NaCl and streaked on appropriate selective media to detect phenotypic expression of each unselected marker after incubation at 37 C for 48 hr. Control cultures for each cross (donor and recipient strains) were streaked on identical plates, as were the recombinants.

Growth of strains and extract preparation for PTS assays. Strains were grown to early stationary phase in 1 liter of medium A with 0.2% lactate plus the required supplements, harvested at 16,300 × *g* in a Sorvall refrigerated centrifuge for 15 min, washed in cold 0.9% NaCl, and centrifuged; the entire pellet (ca. 2 g) was resuspended in 7 ml of 0.01 M potassium phosphate buffer, *p*H 7.5, containing 1 mM disodium (ethylenedinitrilo)tetraacetate (EDTA) and 0.1 mM dithiothreitol (DTT). The cell suspension was passed through an Amicon French pressure cell at 6 to 10 tons and was centrifuged for 10 min at 12,100 × *g* to remove cell debris. The resulting supernatant fluid was centrifuged at 4 C for 2 hr at 226,400 × *g* (Beckman L2-65B, Ti50 rotor), yielding a clear supernatant fluid containing HPr, enzyme I, and factor III. The pellet, containing membrane-bound enzymes II (9), was resuspended with a Teflon-glass tissue homogenizer in 10 ml of the above buffer, centrifuged at 226,400 × *g* for 2 hr, resuspended in 1 ml of buffer, and used without further fractionation.

PTS assays. In general, the procedure for assaying each PTS component was as previously described (1, 8, 9). For assay of one protein of the PTS,

TABLE 1. *Strains of Salmonella typhimurium used in transduction mapping of the cysA-trzA-ptsH-ptsI . . . (crr) gene region*

SB no.[a]	Relevant genotype	PTS defect[b]	Mutagen[c]	Source
	Wild-type LT-2	None		P. E. Hartmen
1475	*ptsH15*	HPr	AP	M Saier (15)
1698	*trzA201 ptsH15*	HPr	NG (*trzA201*)	This paper
1477	*ptsI18*	Enzyme I	AP	M. Saier (15)
2080	*trzA212 ptsI18*	Enzyme I	DES (*trzA212*)	This paper
1675	*ptsI23*	Enzyme I	NG	M. Saier
2077	*trzA209 ptsI23*	Enzyme I	DES (*trzA209*)	This paper
1682	*ptsI19*	Enzyme I	AP	M. Saier
1683	*ptsI20*	Enzyme I	AP	M. Saier
1684	*ptsI21*	Enzyme I	AP	M. Saier
1861	*ptsI26*	Enzyme I	AP	M. Saier
1973	*ptsI29*	Enzyme I	AP	M. Saier
1974	*ptsI30*	Enzyme I	AP	M. Saier
	cysA20	None		P. E. Hartman
1985	*cysA20 ptsI31*	Enzyme I	DES	This paper
1989	*cysA20 ptsI33*	Enzyme I	DES	This paper
2075	*cysA20 ptsH28*	HPr	Recombination[d]	This paper
1465	*purC7 ilv-405*	None		R. Simoni
1469	*ptsI9 purC7 ilv-405*	Enzyme I	NG	R. Simoni
1472	*ptsI13 purC7 ilv-405*	Enzyme I	NG	R. Simoni
SA571	*hisF1009 metA22 trpE2 pyrE231 ilv-99 xyl-1 strA20 malA110*	None		K. Sanderson
1548	SA571, *ptsI40*	Enzyme I	DES	This paper
	trpB223	None		E. Balbinder
1704	*ptsH28 trpB223*	HPr	DES	This paper
1866	*trzA202 ptsH28 trpB223*	HPr	DES (*trzA202*)	This paper
1690	*ptsI34 trpB223*	Enzyme I	DES	This paper
2206	*trzA214 ptsI34 trpB223*	Enzyme I	DES (*trzA214*)	This paper
2226	*ptsH38 trpB223*	HPr	DES	This paper
2227	*ptsI39 trpB223*	Enzyme I	DES	This paper
2309	*trzA-ptsHI41 trpB223*	HPr, enzyme I deletion	NA	This paper
2310	*trzA-ptsHI42 trpB223*	HPr, enzyme I deletion	NA	This paper
2311	*trzA-ptsHI43 trpB223*	HPr, enzyme I deletion	NA	This paper
2313	*trzA-ptsHI44 trpB223*	HPr, enzyme I deletion	NA	This paper
2314	*trzA-ptsHI45 trpB223*	HPr, enzyme I deletion	NA	This paper
2348	*trzA-ptsHI46 trpB223*	HPr, enzyme I deletion	NA	This paper
2349	*trzA-ptsHI47 trpB223*	HPr, enzyme I deletion	NA	This paper
2357	*trzA-ptsHI48 trpB223*	HPr, enzyme I deletion	NA	This paper
2950	*trzA-ptsHI-crr49 trpB223*	HPr, enzyme I, crr (factor III) deletion	NA	This paper
2319	*recA101 hisBH22 strB651*	None		J. H. Wyche
2341	*recA101 hisBH22 strB651*/F'S403	None		J. H. Wyche
SW1403	Hfr H2 *met-469 aro-164 strA H1^b H2^{enx}*	None		H. Mäkelä

[a] Strains are listed in sections with the parent strain at the top of each section.

[b] PTS defects are *ptsH*, HPr; *ptsI*, enzyme I; and *crr*, factor III.

[c] Mutagens are AP, 2-aminopurine; DES, diethylsulfate; NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; NA, nitrous acid.

[d] See footnote to Table 4.

TABLE 2. *Phenotypes of representative strains containing trzA, ptsH, ptsI, and crr defects*

Genotype	Phenotype[a]					
	lac[b]	lac + trz	gal	fru	mtl	mal
LT-2	+	−	+	+	+	+
trpB223	+	−	+	+	+	+
trzA	+	+	+	+	+	+
ptsH	+	−	+	+	−	±
trzA ptsH	+	+	+	+	−	±
ptsI	+	−	+	−	−	−
trzA ptsI	+	+	+	−	−	−
trzA-ptsHI del	+	+	+	−	−	−
trzA-ptsHI-crr del	+	+	+	−	−	+

[a] Strains were grown in nutrient broth overnight and streaked on solid medium for single colonies. Growth was monitored on chemically defined medium (medium A), and fermentation was tested on EMB medium. For sugars, + = growth and fermentation after 48 hr of incubation at 37 C; − = no growth and fermentation under these conditions. For 1,2,4-triazole, + = growth on chemically defined medium (medium A) containing 0.2% lactate plus 10 mM 1,2,4-triazole (±20 μg of L-tryptophan/ml as required) in 48 hr at 37 C; − = no growth under the above conditions. The *ptsH* and *ptsI* point mutations are distinguishable phenotypically because *ptsH* mutants can utilize fructose whereas *ptsI* mutants cannot. A possible explanation for this phenotypic difference has been reported in preliminary form (15).

[b] Abbreviations: lac, lactate; gal, galactose; fru, fructose; mtl, mannitol; mal, maltose; trz, 1,2,4-triazole. All sugars were of the D configuration. When *trpB223* or *cysA20* was in the genetic background of a strain, either L-tryptophan or L-cysteine was added to medium A at a final concentration of 20 μg/ml each. Strains derived from SB1465 were grown in the presence of 20 μg of L-isoleucine, L-valine, and adenosine per ml. SA571 and SB1548 were grown in the presence of 20 μg of L-histidine, L-methionine, L-tryptophan, uracil, L-isoleucine, and L-valine per ml.

the rate of sugar phosphorylation was made directly proportional to the quantity of that protein in the incubation mixture, with the other PTS components present in excess. Either purified HPr (8 μg) or enzyme I (28 μg) was used when the other was assayed, whereas both were added when the enzyme II activities were being determined. The assays for HPr and enzyme I were conducted with membranes prepared from SB1690 (*ptsI34*), SB2309 (*trz-ptsHI41*), or SB2950 (*trzA;ptsHI-crr49*) as the source of enzymes II (0.22 to 0.31 mg of membrane protein per incubation). Each 100-μliter incubation mixture contained the following (in μmoles): potassium phosphate buffer, pH 7.5, 5.0; DTT, 0.25; KF, 0.25; $MgCl_2$, 0.5; PEP, 1.0; and [^{14}C]sugar, 1.0. Methyl α-[^{14}C]glucoside was used in the HPr and enzyme I assays and for the glucose enzymes II determinations; other enzymes II were assayed by use of labeled D-mannose, D-fructose, and D-mannitol. All sugars were used at specific activities of 1.6×10^5 to 2.8×10^5 counts per min per μmole. Factor III was assayed with [^{14}C]TMG at a specific activity of 2.5×10^5 or 3.9×10^5 counts per min per μmole. For the factor III assays, *Salmonella* strains were grown on lactate plus tryptophan, harvested, washed, and prepared as described above, except that the final cell pellets were suspended in 7 ml of 0.025 M Bicine buffer, pH 7.0, containing 1 mM EDTA and 0.2 mM DTT. Incubations were routinely conducted for 30 min at 37 C, and product formation, [^{14}C]sugar-P, was determined by the ion-exchange method (1, 8, 9). Reaction rates were found to be constant during each incubation period. Protein was determined by a biuret procedure (10) with bovine serum albumin as standard; specific activity is expressed as micromoles of sugar phosphate formed per milligram of protein in 30 min at 37 C under the conditions described above.

Reversion and recombination frequencies. The frequency of reversion (Table 6) and of recombination (Tables 3, 4, 5, and 8) are expressed as the actual number of clones detected per 10^8 cells plated. The data in Table 7 were obtained by concentration of the transduction mixture as described in the footnote.

RESULTS

Order of cysA-trzA-ptsH. Preliminary transduction tests showed *ptsH* and *ptsI* mutants to be approximately 46% cotransducible with *cysA20*, a stable deletion of the three *cysA* cistrons (12). Phage grown on two different *trzA ptsH* double mutants were used as donors and *cysA20* was used as the recipient in three-point transduction tests. Figure 1 depicts two possible gene orders. If order I is correct, the minority recombinant class should have the RH$^+$ (*trzA ptsH*$^+$) phenotype; if order II is correct, then the minority recombinant class should have the SH$^-$ (*trzA*$^+$ *ptsH*) phenotype. In fact, SH$^-$ recombinants were far fewer than RH$^+$ recombinants (Table 3, crosses 1 and 2). Thus, the more favored gene order of this region is order II: *cysA-trzA-ptsH*.

Order of cysA-trzA-ptsI. In similar three-point tests (Fig. 2), donor phage was propagated on two different *trzA ptsI* double mutants and used to transduce *cysA20* to prototrophy (Table 3, crosses 3 and 4). In this case, *cysA*$^+$ selection yielded a minority recombinant class which was SI$^-$ (*trzA*$^+$ *ptsI*), suggesting that the relative gene order is *cysA-trzA-ptsI* (Fig. 2, order II). In addition, the data in Table 3 show that the *trzA-ptsH-ptsI* gene region is approximately 46% cotransduc-

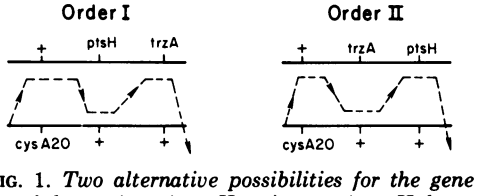


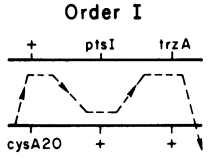
FIG. 1. *Two alternative possibilities for the gene order of the cysA-trzA-ptsH region: trzA ptsH donor × cysA20 recipient.*

TABLE 3. *Three-point tests with cysA⁺ as the selected marker*

Part	Cross no.	Donor × recipient	Recombinant classes				
			RH⁻	SH⁻	RH⁺	SH⁺	Total
A. *trzA ptsH* as the donor fragment[a]	1	*trzA201 ptsH15* × *cysA20*	198	2	67	305	572
	2	*trzA202 ptsH28* × *cysA20*	183	4	53	76	316
			RI⁻	SI⁻	RI⁺	SI⁺	Total
B. *trzA ptsI* as the donor fragment[b]	3	*trzA212 ptsI18* × *cysA20*	220	7	12	223	462
	4	*trzA214 ptsI34* × *cysA20*	177	7	19	205	408

[a] Part A. Transduction was performed on medium A + 0.2% D-fructose selecting only for *cysA*⁺. The *trzA* and *ptsH* mutations were unselected and were detected phenotypically among the recombinants by streaking each transductant on a series of medium A agar plates which were as follows: (i) 0.2% lactate + 10 mM 1,2,4-triazole; (ii) 0.2% D-fructose; and (iii) 0.2% D-mannitol. The four recombinant classes appeared as follows on the three media. RH⁻ (*trzA ptsH*): (i) +, (ii) +, (iii), −. SH⁻ (*trzA*⁺ *ptsH*): (i) −, (ii) +, (iii) −. RH⁺ (*trzA ptsH*⁺): (i) +, (ii) +, (iii) +. SH⁺ (*trzA*⁺ *ptsH*⁺): (i) −, (ii) +, (iii) +.

[b] Part B. Transduction was performed on medium A + 0.2% D-galactose selecting only for *cysA*⁺. The *trzA* and *ptsI* mutations were unselected and were detected phenotypically among the recombinants by streaking each transductant on a series of medium A agar plates which were as follows: (i) 0.2% lactate + 10 mM 1,2,4-triazole; (ii) 0.2% D-galactose; and (iii) 0.2% D-mannitol. The four recombinant classes appeared as follows on the three media. RI⁻ (*trzA ptsI*): (i) +, (ii) +, (iii) −. SI⁻ (*trzA*⁺ *ptsI*): (i) −, (ii) +, (iii) −. RI⁺ (*trzA ptsI*⁺): (i) +, (ii) +, (iii) +. SI⁺ (*trzA*⁺ *ptsI*⁺): (ii) −, (ii) +, (iii) +. Recombinants and control cultures were scored after incubation conditions described in Materials and Methods.



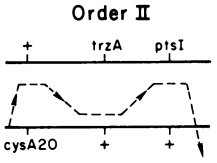
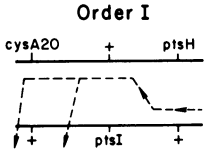


FIG. 2. *Two alternative possibilities for the gene order of the cysA-trzA-ptsI region: trzA ptsI donor × cysA20 recipient.*



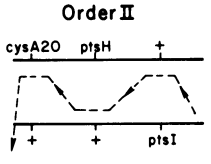


FIG. 3. *Two alternative possibilities for the gene order of the cysA-ptsH-ptsI region: cysA20-ptsH28 donor × ptsI recipient.*

ible (range, 35 to 58%) with *cysA* and that the genetic distance between *cysA* and *trzA* is greater than that separating *trzA* from *ptsH* or *ptsH* from *ptsI*.

Order of cysA-ptsH-ptsI. Phage grown on a *cysA20 ptsH28* double mutant (constructed and verified as described in the footnote to Table 4 and in Table 9) was used as donor in two crosses with different *ptsI* mutants as recipients; in each cross, *ptsI*⁺ was used as the selected marker. With reference to Fig. 3, if order I were correct, then the A⁻H⁺ (*cysA20 ptsH*⁺) recombinants would approximately equal the A⁺H⁺ (*cysA*⁺ *ptsH*⁺) recombinants; if order II is correct, then the A⁻H⁺ recombinants resulting from a quadruple crossover event would be far fewer than the A⁺H⁺ recombinants. The data in Table 4 clearly show that the A⁻H⁺ recombinants constituted the minority class in both crosses, so that the gene order of this region is inferred to be *cysA-ptsH-ptsI*.

The three-point test data presented so far indicate a cumulative gene order of *cysA-trzA-ptsH-ptsI*. To verify the gene order *trzA-ptsH-ptsI*, phage grown on two different *trzA ptsH* double mutants were used to transduce four separate *ptsI* mutants to *ptsI*⁺. If the gene sequence is order I in Fig. 4A, then RH⁺ (*trzA ptsH*⁺) recombinants should approximately equal SH⁺ (*trzA*⁺ *ptsH*⁺) recombinants; if order II is correct, then the RH⁺ recombinants should constitute the minority class. Crosses 1 through 6 in Table 5 show the RH⁺ recombinants to be the minority class, thereby favoring order II: *trzA-ptsH-ptsI*.

Reciprocal three-point tests were performed to substantiate the above conclusion. Phage grown on two *ptsH* mutants were used to infect two *trzA ptsI* double mutants. If order I in Fig. 4B is correct, then again the RH⁺ and SH⁺ recombinants should be approximately equal; if order II is correct, then, this time, the SH⁺ recombinants should constitute the minority class. Crosses 7 through 10 in Table 5 show the SH⁺ recombinants to be in the mi-

TABLE 4. *Three-point tests with donor phage grown on cysA20 ptsH28 and with two ptsI mutants as recipients*[a]

Cross no.	Donor[b] × recipient	Recombinant classes				
		A^-H^-	A^+H^-	A^+H^+	A^-H^+	Total
1	*cysA20 ptsH28* × *ptsI23*	118	398	61	3	580
2	*cysA20 ptsH28* × *ptsI34*	258	359	27	4	648

[a] Transduction was performed on medium A + 0.2% D-fructose + 20 μg of L-cysteine/ml in cross 1; in cross 2, the selective medium contained the above plus 20 μg of L-tryptophan/ml because *ptsI34* contains the *trpB223* mutation in its genetic background. Only *ptsI*$^+$ was selected in both crosses. The *cysA20* and *ptsH28* mutations were unselected and were detected phenotypically among the recombinants by streaking each transductant on a series of medium A agar plates which were as follows: (i) 0.2% D-fructose; (ii) 0.2% D-fructose + 20 μg of L-cysteine/ml; (iii) 0.2% D-mannitol; (iv) 0.2% D-mannitol + 20 μg of L-cysteine/ml. The four recombinant classes appeared as follows on the four media. A^-H^- (*cysA20 ptsH28*): (i) −, (ii) +, (iii) −, (iv) −. A^+H^- (*cysA*$^+$ *ptsH28*): (i) +, (ii) +, (iii) −, (iv) −. A^+H^+ (*cysA*$^+$ *ptsH*$^+$): (i) +, (ii) +, (iii) +, (iv) +. A^-H^+ (*cysA20 ptsH*$^+$): (i) −, (ii) +, (iii) −, (iv) +.

[b] The donor strain used in these three-point tests (SB2075: *cysA20 ptsH28*) was constructed by transduction with SB1989 (*cysA20 ptsI33*) as recipient and phage grown on *ptsH28* as donor. Transduction was performed on medium A containing 0.2% D-fructose + 20 μg of L-cysteine/ml when *ptsI*$^+$ was selected. HPr and enzyme I assays on SB2075 confirmed the presence of the *ptsH28* mutation in the *cysA20* background (see Table 9).

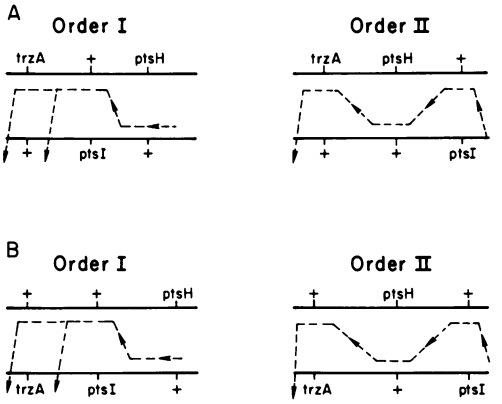


FIG. 4. *Two alternative possibilities for the gene order of the trzA-ptsH-ptsI region: (A) trzA ptsH donor × ptsI recipient; (B) ptsH donor × trzA ptsI recipient.*

nority. This confirms that the gene order is *trzA-ptsH-ptsI*. All of the three-point test data taken together suggest the gene order: *cysA-trzA-ptsH-ptsI*.

Deletions encompassing pts loci. Deletions encompassing *trzA* and extending into the *pts* loci were obtained by a modification of a published procedure for nitrous acid mutagenesis (17). The nitrous acid mutagenesis procedure was identical to the described method (17), except that the reaction was terminated with medium A containing 0.2% lactate and 20 μg of L-tryptophan/ml instead of M63 (salts-buffer), and the cells were resuspended in nutrient broth (Difco) instead of LB broth. A nutrient broth overnight culture of *trpB223* was mutagenized with nitrous acid (17), allowed to grow overnight in nutrient broth, and then plated on medium A containing 0.2% lactate, 20 μg of L-tryptophan/ml, and 10 mM 1,2,4-triazole (LTT plates). Clones appearing after 48 to 72 hr at 37 C were transferred with sterile toothpicks to LTT plates as masters. The masters were incubated overnight at 37 C and replica-plated onto EMB medium containing 1% D-mannitol; white (nonfermenting) subclones were picked. The subclones appeared only at the periphery of mottled clones and were stable when purified through three successive single-colony isolations on the same indicator medium. These putative deletions were observed only as described and never appeared as isolates in which the entire original clone (on the EMB replica plate) was totally nonfermenting. An explanation for these results is presented in the Discussion.

Characterization of pts deletions. The subclones were checked for the presence of the original tryptophan requirement of the parent strain and for 1,2,4-triazole resistance, and were subsequently examined according to the following criteria to determine whether they were extended *pts* deletions: (i) ability to revert spontaneously or in response to potent mutagens on medium A plates containing 20 μg of L-tryptophan/ml and either 0.2% D-mannitol or 0.2% D-fructose as the sole carbon source, (ii) ability to give rise to donor-type recombinants (with D-fructose an the sole carbon source) when used as recipients where phage were propagated on HPr (*ptsH*) point

TABLE 5. *Three-point tests (A) with trzA ptsH double mutants as donors and ptsI mutants as recipients and (B) with ptsH mutants as donors and trzA ptsI mutants as recipients*[a]

Part	Cross no.	Donor × recipient	Recombinant classes				
			RH⁻	SH⁻	RH⁺	SH⁺	Total
A	1	*trzA201 ptsH15* × *ptsI9*	305	17	7	33	362
	2	*trzA201 ptsH15* × *ptsI13*	94	5	0	3	102
	3	*trzA201 ptsH15* × *ptsI23*	359	35	3	28	425
	4	*trzA201 ptsH15* × *ptsI34*	663	16	4	19	702
	5	*trzA202 ptsH28* × *ptsI23*	187	28	3	33	251
	6	*trzA202 ptsH28* × *ptsI34*	469	20	6	20	515
B	7	*ptsH15* × *trzA209 ptsI23*	17	179	7	1	204
	8	*ptsH15* × *trzA214 ptsI34*	17	315	7	0	339
	9	*ptsH28* × *trzA209 ptsI23*	54	517	37	7	615
	10	*ptsH28* × *trzA214 ptsI34*	6	149	8	1	164

[a] Transduction was performed on medium A + 0.2% D-fructose selecting only for *ptsI*⁺. In part A, L-isoleucine, L-valine, and adenosine were added to the agar at a final concentration of 20 μg/ml each for transduction and the analysis of recombinants derived from *ptsI9* and *ptsI13*. In both parts A and B, 20 μg of L-tryptophan/ml was added to the agar when *ptsI34* was the recipient. All recombinants from each transduction in part B were first purified through one single-colony isolation on the original selection medium before streaking on the selective media. All four recombinant classes were detected phenotypically among the transductants by streaking on the media described in footnote *a* of Table 3.

mutations, (iii) ability to recombine with known HPr (*ptsH*) and enzyme I (*ptsI*) point mutations on medium A-tryptophan plates containing 0.2% D-mannitol, and (iv) presence or absence of HPr, enzyme I, and factor III activities in the in vitro PTS assays.

Table 6 demonstrates the nonrevertability of nine presumed deletions isolated from *trpB223* in comparison with known *ptsH* and *ptsI* point mutations in response to two mutagens highly effective in causing base substitutions in *Salmonella*. Table 7 shows that when phage grown on three *ptsH* point mutants were crossed into each of the nine deletions as recipients only one recombinant class was recoverable, namely, donor type. Within the limits of our tests (see Table 7), all deletions include each *ptsH* mutant examined (see also data in

TABLE 6. *Reversion patterns of PTS point mutants and deletions*[a]

Part	SB no.	PTS defect	Reversion on selective medium[b]		
			DES	NG	Spontaneous
A. *pts* point mutants	1475	*ptsH15*	1,000	500	100
	1704	*ptsH28*	1,000	200	20
	1989	*ptsI33*	8	0	0
	1690	*ptsI34*	9	3	2
	1469	*ptsI9*	3	3	0
	1472	*ptsI13*	4	3	0
B. *pts* deletions	2309	*trzA-ptsHI41*	0	0	0
	2310	*trzA-ptsHI42*	0	0	0
	2311	*trzA-ptsHI43*	0	0	0
	2313	*trzA-ptsHI44*	0	0	0
	2314	*trzA-ptsHI45*	0	0	0
	2348	*trzA-ptsHI46*	0	0	0
	2349	*trzA-ptsHI47*	0	0	0
	2357	*trzA-ptsHI48*	0	0	0
	2950	*trzA-ptsHI-crr49*	0	0	0

[a] Each strain containing a *pts* mutation was grown overnight in nutrient broth. Amounts of 0.1 ml (ca. 10^8 bacteria) of each culture were spread onto medium A plates (with the required supplements) plus 0.2% D-mannitol (in parts A and B) and 0.2% D-fructose (part B). The data represent the number of revertants appearing on selective medium after mutagenesis and incubation for 5 days at 37 C.

[b] The mutagens used were diethylsulfate (DES) and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG).

TABLE 7. *Recombination with phage carrying ptsH point mutations when pts deletions were used as recipients*[a]

pts deletion recipients	Donor (*ptsH*) recombinants/ total *ptsH* donors		
	ptsH15	*ptsH28*	*ptsH38*
trzA-ptsHI41	126/126	76/76	96/96
trzA-ptsHI42	48/48	58/58	56/56
trzA-ptsHI43	101/101	117/117	110/110
trzA-ptsHI44	35/35	144/144	136/136
trzA-ptsHI45	83/83	80/80	104/104
trzA-ptsHI46	10/10	32/32	32/32
trzA-ptsHI47	31/31	49/49	82/82
trzA-ptsHI48	15/15	26/26	45/45
trzA-ptsHI-crr49	ND[b]	ND	ND

[a] Phage P22 grown on donor strains carrying *ptsH* point mutations were used to transduce nine deletions of the *pts* region. The transduction mixtures were then concentrated 10-fold by centrifugation and plated on medium A agar plates containing 0.2% D-fructose plus 20 μg of L-tryptophan/ml. After 48 hr at 37 C, the transductants were streaked onto a series of medium A plates containing 0.2% D-fructose, 0.2% D-mannitol, or 0.2% lactate plus 10 mM 1,2,4-triazole. All plates contained 20 μg of L-tryptophan/ml. No prototrophic recombinants appeared in any cross; all recombinants showed the *ptsH* (*trzA*$^+$ *ptsH*) phenotype of the donors (see Table 2 and footnotes to Table 3). In addition, the recombinants were streaked onto EMB agar plates containing either 1% D-fructose or 1% D-mannitol. Growth and fermentation tests consistently gave identical results.

[b] Not done.

Table 8 for corroboration). Table 8 demonstrates the recombination frequencies of the nine *pts* deletions with a variety of *ptsH* and *ptsI* mutations under conditions where only prototrophic recombinants are selected. From the approximate recombination frequencies between donor phage carrying *ptsI* point mutations and the recipient deletions, the relative termination point of each *pts* deletion into the *ptsI* gene can be estimated. Since the *pts* deletion in SB2950 fails to recombine with the most distal *ptsI* point mutation tested (*ptsI40*), this deletion probably extends through the entire *ptsI* gene and into or through the *crr* gene. Figure 5 represents a deletion map of the *pts* region derived from the results in Table 8.

Table 9 gives the results of assays for the PTS proteins in extracts of all *pts* deletions and for several *ptsH* and *ptsI* point mutations. All deletions lack both HPr and enzyme I activities, whereas *ptsH* or *ptsI* point mutations contain one or the other activity. Even the strongly polar HPr mutants, *ptsH28* and *ptsH15*, contain detectable enzyme I activity. We conclude that the nine *pts* defects represent extended deletions of a portion or all of the *trzA* gene including the entire *ptsH* gene. Eight of the nine *pts* deletions end before the distal boundary of *ptsI*. As yet, no deletions have been recovered which include only *trzA* and *ptsH*, leaving the entire *ptsI* gene intact. Table 9 also shows that the *pts* deletions contain either normal or elevated levels of the

TABLE 8. *Recombination with phage grown on ptsH and ptsI point mutations when pts deletions were used as recipients*[a]

SB no.	*pts* deletion recipients	*ptsH* and *ptsI* donors															
		H15	H28	H38	I18	I19	I33	I34	I39	I26	I29	I20	I21	I23	I30	I31	I40
2310	*trzA-ptsHI42*	0	0	0	0	0	0	0	0	18	33	205	162	221	250	500	1,500
2311	*trzA-ptsHI43*	0	0	0	0	0	0	0	0	10	2	33	25	25	22	70	450
2313	*trzA-ptsHI44*	0	0	0	0	0	0	0	0	10	1	48	50	36	66	72	175
2314	*trzA-ptsHI45*	0	0	0	0	0	0	0	0	15	4	72	25	42	46	115	500
2357	*trzA-ptsHI48*	0	0	0	0	0	0	0	0	2	1	5	11	7	12	11	100
2309	*trzA-ptsHI41*	0	0	0	0	0	0	0	0	0	6	22	27	21	65	60	200
2348	*trzA-ptsHI46*	0	0	0	0	0	0	0	0	0	0	2	4	12	16	46	290
2349	*trzA-ptsHI47*	0	0	0	0	0	0	0	0	0	0	0	8	14	13	28	172
2950	*trzA-ptsHI-crr49*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] Phage KB1 grown on strains carrying *ptsH* and *ptsI* point mutations were used to transduce nine *pts* deletions. The transduction mixtures were spread onto medium A agar plates containing 0.2% D-mannitol and 20 μg of L-tryptophan/ml, allowing for the selection of prototrophic recombinants only. After 48 to 72 hr of incubation at 37 C, the transductants were scored on medium A plates containing 0.2% lactate plus 10 mM 1,2,4-triazole, on medium A plates containing 0.2% D-mannitol, and on EMB medium containing 1% D-mannitol. All medium A plates contained 20 μg of L-tryptophan/ml. All recombinants showed the wild-type phenotype (*trzA*$^+$ *pts*$^+$; see Table 2 and footnotes to Table 3).

[b] Number of prototrophic recombinants.

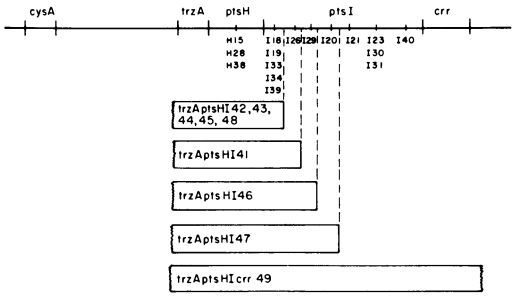


FIG. 5. *Deletion map of the pts gene region in Salmonella typhimurium.*

enzymes II required for the transfer of the phosphoryl group from phospho-HPr to methyl α-glucoside (a glucose analogue), mannose, fructose, or mannitol; these results place the structural genes for these enzymes II outside the *pts* deletions encompassing the genetic region from *trzA* to *crr*.

The phenotype of the *pts* deletion in SB2950 corresponds to a class of mutant strains designated *ptsI crr* (14). In contrast to the other *pts* deletions, it can utilize maltose. Since the *crr* phenotype is thought to be associated with a mutation in a sugar-specific protein of the PTS, factor III, assays of all *pts* deletion extracts revealed that only SB2950 was totally lacking detectable factor III activity (Table 9). This observation agrees with the genetic data and the phenotype of SB2950.

F′ carrying pts loci. SB2341 (*recA101 hisBH22 strB651*/F′S403) was constructed by mating an *S. abony* Hfr (SW1403: Hfr H2 *met-469 aro-164 strA H1*^{b} *H2*^{enx}) with SB2319 (*recA101 hisBH22 strB651*) on appropriate selective media (Wyche et al., Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 43, 1972).

The F′ in SB2341 was shown to include the *pts* region by crosses with *ptsI34* (Fig. 5) and the *pts* deletion in SB2309 (Table 1, Fig. 5) on medium A plates containing 20 μg of L-tryptophan/ml and 0.2% D-mannitol (Wyche et al., Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 43, 1972). In addition, the episome-containing strain produced three to six times as much HPr and enzyme I as did either parent (Table 10). Since the F′ carries only genes near *cysA*, this is additional evidence that both *ptsH* and *ptsI* are in this chromosomal region. The data in Table 10 also show that there was no increase in the constitutive enzymes II, which suggests that the episome does not include any enzyme II structural genes.

Transduction and segregation experiments with pts deletions. As described in the section "Deletions encompassing *pts* loci," *pts* deletions were isolated only as subclones at the periphery of mottled clones containing fermenting and nonfermenting cells. This observation led to the idea that these *pts* deletions were recovered as "segregants" of a wild-type cell; that is, nitrous acid mutagenesis resulted in excision, followed by transient replication, and, finally, loss of the excised portion of the genome. An extension of this idea is that recombination performed with a deletion as the recipient operates by a similar mechanism which is the reverse of deletion formation. That is, the intermediate situation between a deletion and a stable recombinant is a cell containing an "episome-like" particle (introduced by the phage) which can be either stably integrated or lost after subsequent cell division. The results of a reconstruction experiment designed to test this idea are presented in Fig. 6. The experiment was as follows: phage P22 L-7, grown on *trpB223*, was used to infect SB2309 containing a *pts* deletion under conditions in which prototrophic (*trzA*$^{+}$-*pts*$^{+}$) transductants are selected. Streaking these transductants on fructose medium first, followed by testing them on EMB, revealed only stable prototrophic recombinants. However streaking these prototrophic transductants directly onto lactate-triazole medium gave rise to a few clones (*trzA* phenotype) after 72 hr at 37 C; these clones, tested by a streaking on EMB fructose, resulted in both positive (*pts*$^{+}$) and negative (*pts*$^{-}$) segregants. Three alternate passages of each transductant, both on fructose and on lactate-triazole medium, gave identical results (see Fig. 6).

These results indicate that there exist at least two populations of cells within a transductant clone: one cell type, constituting the majority of cells, are stable recombinants; a second, minority type which are unstable either can form stable recombinants or can segregate out the *pts*$^{+}$ determinant, resulting in recovery of the deletion. If the clones which appeared on lactate-triazole medium were simply abortive transductants (in which the donor piece had already segregated out), then only nonfermenting clones would have been observed. Analogous to the phenomenon we observed, "pseudo-recombinants" which segregate out the original deletion recipient when transductants are retested on selective medium have been observed in the *trp* operon of *Salmonella* (L. LaScolea and E. Balbinder, *personal communication*). An interpretation of these results is given below.

TABLE 9. *Specific activities of PTS components in various mutant strains*

SB no.[b]	Relevant genotype	PTS components: specific activity						
		HPr	Enzyme I	Enzymes II[c]				Factor III[d]
				α-MG	Man	Fru	Mtl	
	Wildtype LT-2	0.38	0.56	0.13	0.14			
1475	*ptsH15*	<0.01	0.08	0.19	0.22			
1675	*ptsI23*	0.31	<0.01	0.06	0.09			
	cysA20	0.34	0.48	0.10	0.12	0.10	0.05	
1989	*cysA20 ptsI33*	0.66	<0.01	2.2				
2075	*cysA20 ptsH28*	<0.01	0.05	0.14	0.12	0.10	0.05	
1465	*purC7 ilv-405*	0.27	0.52	0.07	0.08			
1469	*ptsI9 purC7 ilv-405*	0.33	<0.01	0.26	0.35			
1472	*ptsI13 purC7 ilv-405*	0.46	<0.01	0.26	0.40			
SA571	*hisF1009 metA22 trpE2 pyrE231 ilv-99 xyl-1 strA20 malA110*	0.48	0.56	0.07	0.14	0.18	0.12	
1548	SA571 with *ptsI40*	0.52	<0.01	0.20				
	trpB223	0.76	2.2	0.54	1.0	0.61	0.29	0.41
1704	*ptsH28 trpB223*	0.01	0.04	0.25		0.13	0.17	
1690	*ptsI34 trpB223*	0.76	<0.01	2.7				
2226	*ptsH38 trpB223*	0.02	1.6	1.1	1.4	1.2	0.50	
2227	*ptsI39 trpB223*	0.80	<0.01	3.5	2.9	3.7	0.71	
2309	*trzAptsHI41 trpB233*	<0.01	<0.01	0.84	0.84	0.57	0.56	0.31
2310	*trzAptsHI42 trpB223*	<0.01	<0.01	0.62	0.79	0.50	0.45	0.30
2311	*trzAptsHI43 trpB223*	<0.01	<0.01	0.90	1.0	0.64	0.54	0.28
2313	*trzAptsHI44 trpB223*	<0.01	<0.01	0.74	0.81	0.79	1.0	0.42
2314	*trzAptsHI45 trpB223*	<0.01	<0.01	0.92	0.98	0.72	0.58	0.38
2348	*trzAptsHI46 trpB223*	<0.01	<0.01	0.61	0.60			0.48
2349	*trzAptsHI47 trpB223*	<0.01	<0.01	0.96	0.89			0.39
2357	*trzAptsHI48 trpB223*	<0.01	<0.01	0.85				0.85
2950	*trzAptsHIcrr49 trpB223*	<0.01	<0.01	0.70	0.90	0.62	0.70	<0.01

[a] Extracts were prepared and PTS assays were performed as described in Materials and Methods. Protein concentration was determined by use of the biuret reagent with bovine serum albumin as the standard. The specific activity of each PTS component is expressed as micromoles of sugar phosphate formed in 30 min at 37 C per milligram of protein. Duplicate determinations on the same extract and on different batches of cells of the same strain showed a 10 to 15% maximal variability.

[b] Strains are arranged in sections with the parent strain at the top of each section.

[c] α-MG, methyl α-D-glucopyranoside; Man, mannose; Fru, fructose; Mtl, mannitol.

[d] Factor III assays are expressed as micromoles of thiomethyl β-D-galactopyranoside phosphorylated per milligram of protein above the control values of the incubation mixture from which factor III has been omitted.

DISCUSSION

The structural genes that code for HPr (*ptsH*) and enzyme I (*ptsI*) of the phosphoenolpyruvate:sugar PTS in *S. typhimurium* are linked to each other and to *cysA* and *trzA* such that the gene order of this region from left to right is *cysA-trzA-ptsH-ptsI*. This conclusion is derived from results of reciprocal three-point tests (Fig. 1–4, Tables 3–5), deletion mapping (Fig. 5, Tables 7 and 8), coordinate increase in both HPr and enzyme I activities in an episome-carrying strain (Table 10), and the polarity expressed by some HPr (*ptsH*) point mutations on enzyme I production from *ptsI* (15; Table 9). Assuming that the results of a single reciprocal three-point test performed by Epstein, Jewett, and Fox (6) reflect the real gene order in *Escherichia* as (*purC*)...*ptsI-ptsH-supN*, the orientation of *ptsH* and *ptsI* in *Escherichia* appears inverted with respect to the *Salmonella* chromosome. The conclusion reached earlier (11), which placed the *ptsI* (*carA*) gene between *azi* and *pro* on the *Salmonella* chromosome, is now thought to have resulted from the existence of a suppressor mu-

TABLE 10. *Specific activities of PTS components in two pts⁺ haploids and a pts⁺/F'S403 pts⁺ merodiploid*[a]

Strain no.	Relevant genotype	PTS components: specific activities					
		HPr	Enzyme I	Enzymes II[b]			
				α-MG	Man	Fru	Mtl
SW1403	*recA*⁺ Hfr H2 *pts*⁺	0.49	0.85	0.08			
SB2319	*recA101 pts*⁺	0.86	1.7	0.05	0.09	0.06	0.08
SB2341	*recA101 pts*⁺/F'S403 *pts*⁺	3.2	5.1	0.06	0.13	0.07	0.10

[a] Strains were grown as described in Materials and Methods with 0.2% lactate as the sole carbon source. L-Histidine was added at a final concentration of 20 μg/ml for growth of SB2319 and SB2341; thiamine and nicotinic acid were present at 5 μg/ml each for the growth of SB2319. SW1403 was grown in the presence of 20 μg each of L-methionine, L-phenylalanine, L-tyrosine, and L-tryptophan per ml. Harvesting and washing of cells and preparation of extracts were as described previously. The source of enzymes II for the HPr and enzyme I assays was a washed membrane preparation from SB2309.

[b] α-MG, methyl α-D-glucopyranoside; Man, mannose; Fru, fructose; Mtl, mannitol.

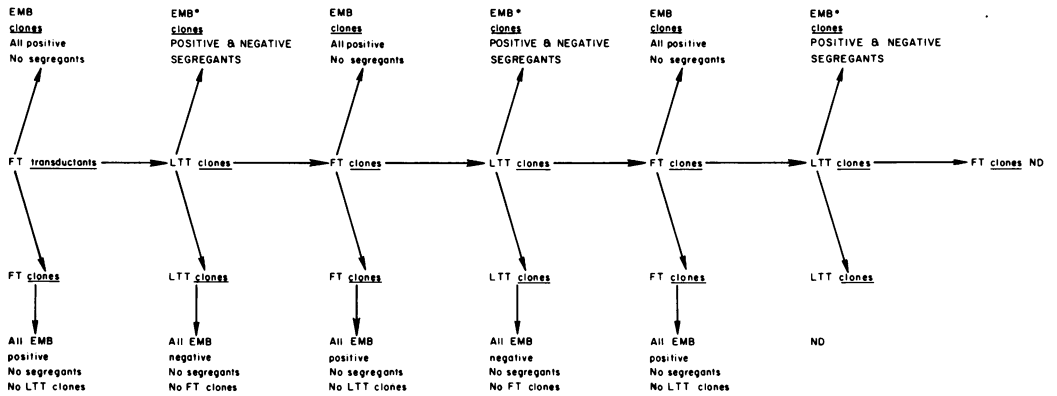


FIG. 6. *Reconstruction experiment with pts⁺ phage and the pts deletion in SB2309. Transduction was performed as previously described with P22 mutant int-7 grown on trpB223 as donor and SB2309 as recipient. Selection for pts⁺ transductants was done on medium A plates containing 0.2% D-fructose and 20 μg of L-tryptophan/ml (FT). EMB contained 1% D-fructose so that pts⁺ segregation could be monitored from the lactate-triazole-tryptophan (LTT) clones. Incubation of each step from original transduction through final scoring of transductants was performed for 48 to 72 hr at 37 C. Although the vast majority of EMB clones streaked from LTT plates (marked with asterisks) contained positive and negative segregants, a few were totally negative, presumably owing to complete segregation of the pts⁺ determinant. ND = not done.*

tation in the *pro* region of the Hfr donor strain employed in conjugational mapping (M. Levinthal, *personal communication*).

From the genetic data establishing close linkage between the gene for 1,2,4-triazole sensitivity (*trzA*) and the structural genes for HPr (*ptsH*) and enzyme I (*ptsI*), the following assumptions were made for *pts* deletion isolation: (i) resistance to 1,2,4-triazole results from the *absence* of a functional *trzA* gene, (ii) there is no unknown gene(s) between *trzA* and the *pts* region which would be lethal if deleted, and (iii) a *ptsH*, *ptsHI*, or *ptsHI-crr* deletion would not itself be lethal. If all of these assumptions are correct, then nitrous acid mutagenesis followed by selection for 1,2,4-triazole resistance (under conditions where functional *pts* genes are unselected, e.g., with lactate as sole carbon source) should result in the recovery of *trzA* deletions extending into or through the *pts* region.

None of the nine *pts* deletions isolated during this study has been characterized genetically to determine whether any of them include the entire *trzA* gene. Eight of the nine deletions have their distal boundaries or termination points within *ptsI*. From the fact that all *ptsH* mutants tested map inside each deletion and that some *ptsI* mutants map outside the distal termini of eight *pts* deletions, the gene order must be *trzA-ptsH-ptsI*, in agreement with the three-point tests. The deletions all contained normal or elevated levels of the sugar-specific enzymes II required for the

phosphorylation of glucose, mannose, fructose, and mannitol, showing that the structural genes for these enzymes II cannot be located between *trzA* and *crr*. Experiments have been performed with an F′ episome, F′S403, that covers *ptsI34* and the terminus of the *pts* deletion in SB2309. Since F′S403 includes *purG* but ends before *aroD* (J. Wyche, *personal communication*), *ptsI* is the last known locus included in the episome. Preliminary assays for factor III show no increase in this activity, which suggests that the *crr* gene is absent from F′S403.

Some *ptsH* point mutations (e.g., *ptsH28* and *ptsH15*) appear to have a "polar" effect on enzyme I production from *ptsI* (Table 9; 15), whereas *ptsH38* does not. No *ptsI* mutation isolated to date exerts polarity on HPr production from *ptsH* (Table 9). This suggests the possibility that HPr and enzyme I are translated from the same messenger ribonucleic acid such that the direction of transcription and translation would be from the vicinity of *trzA* with a *pts* "promoter" site between *trzA* and *ptsH*. From the cotransducibility of the HPr and enzyme I genes, the isolation of *pts* deletions, the polarity that some *ptsH* mutants have on *ptsI*, and experiments showing that HPr and enzyme I are coordinately inducible (15), the notion that the *ptsHI* gene cluster constitutes an operon is increasingly attractive.

The *pts* deletion in SB2950 is the longest deletion of this region isolated to date. In addition to eliciting triazole resistance, this deletion lacks the ability to recombine with any known *pts* point mutation, totally lacks any detectable HPr, enzyme I, and factor III activities (see Table 9), and exhibits the *crr* phenotype. These observations suggest that this deletion extends into or through the *trzA* gene (on the left), entirely includes the *ptsH* and *ptsI* genes, and has its terminus either within or beyond the *crr* gene. This agrees with the cotransduction of *crr* with the *ptsHI* gene region (14).

Two major goals of these studies were achieved: (i) to map accurately the structural genes coding for two general proteins (HPr and enzyme I) of the *Salmonella* PTS, and (ii) to provide *pts* deletions for use in accurately defining the physiological functions of the PTS. The availability of *pts* deletions should permit definitive studies on the role these proteins play in cell physiology, sugar transport, enzyme induction, and the regulation of intracellular cyclic adenosine monophosphate concentration.

In addition, we suggest that deletion formation may occur in a fashion similar to the Campbell model for lambda prophage excision (4) and that these *pts* deletions might be used as a model system to explore this phenomenon. From the original appearance of these *pts* deletions as nonfermenting subclones at the periphery of mottled clones and from the behavior of the prototrophic transductants after single-colony isolation on lactate-triazole medium in the reconstruction experiment (Fig. 6), deletion formation may occur by excision of a portion of the chromosome followed by transient or limited replication of the fragment which is generated; dilution of this fragment after subsequent cell division would result in a deletion. Since most deletion isolation procedures are performed under conditions of direct selection, this process would be completed before it could be observed.

ACKNOWLEDGMENTS

We express our gratitude to P. E. Hartman for helpful discussion and to Geraldine Chester for her supervision of the preparation room and sterile facilities. It is a pleasure to acknowledge the expert assistance of E. Wayne Grogan and R. Philip Anderson.

J.C.C. is a Postdoctoral Fellow of the Arthritis Foundation. This work, supported by Public Health Service grant AM-09851 from the National Institute of Arthritis and Metabolic Diseases, grant NP-16A from the American Cancer Society, and a grant from the National Cystic Fibrosis Research Foundation, constitutes Contribution No. 691 from the McCollum-Pratt Institute of the Johns Hopkins University.

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