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Transduction by Phage P1CM *clr*-100 in *Salmonella typhimurium*

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Summary. Phage P1 does not adsorb to *S. typhinurium* wild type cells. It does adsorb to rough derivatives including strains with mutations in the *galE* gene. Phage strain P1CM *clr-100* can be efficiently propagated in *S. typhimurium* derivatives, either by induction of a lysogen, or by lytic infection.

Phage P1 lysates are able to mobilize genetic markers in a generalized fashion. The transduction system is essentially identical to that in *Escherichia coli*, except that $CaCl_2$ is not required for efficient adsorption.

Two regions of the *S. typhimurium* chromosome were mapped by P1-mediated transduction. Several examples of genes linked by P1, and unlinked by P22, are presented. The relative efficiency of P1 over P22 in transduction was not determined, however.

Data presented indicate unambigously that the gene order for the *trp* region is:

his ... dadA–hemA–trp–pyrF ... pyrC

but known markers in between were not used. The gene order for the *cysA* region was determined to be as follows:

pheA ... purC–cysA–trzA–pts–dsd–aroD–purF ... his,

and special mapping problems for this region are discussed.

Introduction

The general transducing phage P1 does not adsorb to *S. typhimurium* LT2 cells. Okada and Watanabe (1968a, b) and more recently Ornellas and Stocker (1974) and Enomoto and Stocker (1974), found that P1 plated effeciently on, and mobilized chromosomal markers to and from, some rough derivatives of S. *typhimurium* LT2.

This paper reports: (i) The conditions for growth of P1 on rough strains of *S. typhimurium*. (ii) Rapid isolation of P1-sensitive *S. typhimurium* strains. (iii) The transduction parameters and (iv) Transduction data for two regions of the *S. typhimurium* chromosome; the *trp* region and the *cysA* region.

Material and Methods

1. Bacterial Strains used are described in Table 1.

2. The following phage strains were used: P22.L4 (Smith and Levine, 1967) deficient in maintenance of lysogeny, and P22.H5 a clear mutant of P22. Phage FO (Kallings, 1967), and C21 (Shedlovsky and Brenner, 1963). The latter two phages were received through the courtesy of Jan Neuhard. Coliphage P1CM *clr-100* is a derivative of P1kc, carrying the CM (chloramphenicol-resistance) marker from R19 drug resistance transfer factor (Kondo and Mitsuhashi, 1964) and a temperature-sensitive repressor (Rossner, 1972). All P1CM *clr-100* stocks used come from a single plaque recovered from the supernatant of strain TR2298 and plated on SL3684. Phage stocks were renewed periodically after single-plaque reisolation.

3. The following media were used: Hut plates (Smith, 1971) for selection of *hut*$^+$ recombinants. LB broth (Lennox, 1955) was the nutrient broth. LA was LB with 1.2% Difco Bacto

Table 1. Bacterial strains

Strain	Parent	Characteristics	Source or reference
LT2		*wild type*	B.N.Ames
SL3684	LT2	*hut⁺ galE503*	Ornellas and Stocker (1974)
TR2298	SL3684	*hut⁺ galE503 (P1CM clr-100)*	J.R.Roth
MA 12	dsd-2	*dsd-2 hut⁺ galE503*	T.Klopotowski
13	purC2	*purC2 hut⁺ galE503*	J.S.Gots
14	TK162	*his203 trzA2 hut⁺ galE503*	T.Klopotowski
15	trpE95	*trpE95 hut⁺ galE503*	E.Balbinder
16	leu32 aroD166	*leu32 aroD166 galE1571*	T.Klopotowski
17	TK4	*aro601 hisT1504 his2323 hut⁺ galE503*	T.Klopotowski
18	TK604	*his3501 dhuA1 dadA1 hut⁺ galE503*	Wild *et al.* (1973)
19	pyrF146	*pyrF146 hut⁺ galE503*	K.E.Sanderson
20	pyrC138	*pyrC138 hut⁺ galE503*	K.E.Sanderson
21	TR114	*aroD5 hisD23 galE1572*	J.R.Roth
22	TK672	*his3501 dhuA1 ilvA5 hemA205 hut⁺ galE503*	Wild *et al.* (1973)
23	SHSM70	*hemA70 hut⁺ galE503*	Sasarman *et al.* (1970)
24	TK639	*his612 dhuA1 dadA1 amtA80 pyrF146 galE1573*	T.Klopotowski
25	SB2950	*trzA ptsHI crr49 (Δ) trpB223 hut⁺ galE503*	Cordaro and Roseman (1972)
26	SB2206	*trzA214 ptsI34 trpB223 hut⁺ galE503*	Cordaro and Roseman (1972)
27	MA22	*his3501 dhuA1 ilvA5 trpE95 hut⁺ galE503*	MA22 × P1.MA15 (hemA⁺ trp)
28	TK512	*purF145 his3501 hut⁺ galE503*	T.Klopotowski
32	purB12	*purB12 galE1575*	J.S.Gots
34	cysA20	*cysA20 galE1577*	D.A.Smith
38	aroA1	*aroA1 galE1579*	T.Klopotowski
39	aroE88	*aroE88 hut⁺ galE503*	T.Klopotowski
42	cysB403	*cysB403 galE1600*	K.E.Sanderson
45	SU687	*trpA52 pyrF146 galE1580*	K.E.Sanderson
73	4420	*metA metE trpB str-r val* r^-_{LT} m^+_{LT} r^-_S m^+_S *galE1585*	C.Colson
77	TR248	*cysA1348 hisC527 supZ501 galE1588*	J-R.Roth
82	SL3684	*hut⁺ galE503 (P1CM clr-100)*	*a*
111	MA77	*cysA1348 hisC527 galE1588 hemA321*	*b*

All strains are derivatives of *S. typhimurium* strain LT2, except MA45 which is a derivative of strain LT7. All *hut⁺ galE503* were generated as *hut⁺* transductants with SL3684 as donor. Other *galE* strains were generated as resistant to phage FO as described in the text. Source refers to the parent strain. See Sanderson (1972) for key to genetic notations.

[a] Chlorampenicol resistant "transductant".

[b] Neomycin-resistant mutant isolated as described by Sasarman *et al.* (1970).

Agar. LSA was LB with 0.65% Difco Bacto Agar. LACM was LA with 12.5 μg chloramphenicol per ml. E and NC plates were as described by Wild *et al.* (1973).

4. Bacteriophage techniques were as described by Eisenstark (1967) and for P1 as described by Scott (1968).

5. Mutants resistant to phages FO or P22 were obtained by spreading 0.1 ml cells and about 10^{10} p.f.u. of either phage on LA. After overnight incubation at 37° plates were scored and single colonies stabbed onto LA plates. After overnight incubation each colony was tested for phage sensitivity, galactose utilization and parent-strain markers. Strains with mutations in the *galE* gene are brittle, dry, and break on contact with a needle.

6. CM-resistant mutants (lysogens) were obtained by mixing 0.5 ml of cells with 0.2 ml of P1CM *clr 100* (to desired m.o.i.). After 20 min incubation at 37°, aliquots were plated on LACM plates and scored after 48 h incubation at 30°. Colonies were tested for phage and temperature sensitivity, galactose utilization, parent-strain markers and P1 release. Spontaneous

P1 release was detected by plating sterile cell suspension on MA73 at 42°. When required, P1-lysogens generated this way, were cured by transferring five times at 42° on LA plates.

7. Transduction with P1 was performed as follows:

a) Recipients in transduction were grown overnight (ON) at 37° without shaking. Under these conditions, cell titers varied between 1.3×10^9 and 3.0×10^9 colony forming units per ml.

b) P1 was grown by temperature induction of appropriate lysogens or by lytic infection of donor strains.

c) The standard transduction procedure consisted of mixing 0.5 ml of recipient cells with 0.5 ml of phage of appropriate dilutions to give m.o.i. between 1.0 and 10. Mixture was incubated for 10 min at 37°. After vortexing, 0.25 ml aliquots per plate were spread on selective plates, which were incubated at 30° for 48 h and scored. Variations of the standard transduction procedure included: m.o.i. incubation length and temperature of mixture before plating, presence of $CaCl_2$ in the mixture and temperature of incubation of transduction plates. Controls included: P1 alone on B plates and on selective plates, recipient alone or a mixture of recipient and phage previously propagated on recipient.

d) Selection was on E plates supplemented with unselected donor and recipient requirements (if any); except that selection for *hem* A^+ was always carried out on LA plates (Sasarman *et al.*, 1970), and selection for *pts*$^+$ was carried as described by Cordaro and Roseman (1972). The *amt* marker was always unselected and scored as described by Wiater and Klopotowski (1972).

All experiments involving *dsd* were performed at 37°, since scoring of the *dsd* phenotype at 30° is ambiguous; *dsd* was always the unselected marker, since it is not feasible to select for *dsd*$^+$. Each recombinant was tested individually, for DL-serine sensitivity, by plating 0.1 ml of ON cells, grown in supplemented minimal medium, in 3 ml soft agar, and applying a crystal of DL-serine in the center of the plate, since replica-plating was not adequate and cell growth on B medium partially masked the *dsd* phenotype. Strains carrying the *dsd-2* gene showed a clear zone of inhibition around the crystal of DL-serine. Recombinants carrying *dsd-2* grew poorly, and segregation of the *dsd-2* marker occurred at high frequency, making scoring difficult and data unreliable.

Al recombinants carrying unselected markers were tested twice by replica plating, for the presence of unselected donor markers and other markers, if any; afterwards they were streaked for single cell and retested.

In several cases it was not possible to perform reciprocal crosses, because of failure to obtain good phage titers with certain donors.

e) Traits of P1 lysogens arising after transduction were tested as follows: Recombinant colonies from selective plates were transferred to 2.0 ml of LB and incubated without shaking at 30° for 4 to 6 h. Two LA plates and one LACM plate were spotted with cells. One LA plate was incubated at 42°, the other LA plate and the LACM plate were incubated at 30°. After ON incubation, plates were scored. In addition, 0.2 ml samples were pipeted into 2.0 ml of LB and incubated at 42° for 60 to 120 min, sterilized with chloroform and plated on SL3684 to detect spontaneous P1 release. Growth on LACM plates, no growth on LA plates at 42° and growth at 30°, and spontaneously released P1 particles indicated that the recombinant tested was a P1 lysogen. Otherwise, the recombinant was deemed to be not lysogenic.

Results

1. Sensitivity of S. typhimurium to Coliphage P1. Using a stock of P1CM *clr-100* propagated on a *galE* derivative of LT2 (SL3684), so as to be appropriately modified, the sensitivity of a variety of *S. typhimurium* LT2 derivatives to phage P1 was tested by several methods. The wild type strain (i.e. smooth) was not sensitive to P1 because of failure to adsorb the phage since: a) nearly 100% imput P1 was recovered free (not adsorbed) after 20 min. b) Cell survival was about 100% in the presence of P1 (m.o.i.'s up to 100) and c) The titer of P1 did not increase after prolonged incubation with or without P22 helper. A variety of strains, with cell wall defects, were found to be sensitive to P1 with varying effi-

Table 2. Characteristics of spontaneous FO and P22 resistant clones and PICM *clr-100* mediated chloramphenicol resistant clones of *S. typhimuriun* LT2

Lysis by phages					*gal* phenotype	Frequency (%) after selection for resistance to		
P1	C21	FO	H5	H5gal		FO	H5	CM
+	+	−	−	+	−	8	4	16
+	+	−	−	−	−	3	2	5
+	−	−	−	−	+	3	10	13
+	−	+	−	−	+	<1	2	27
+	−	−	+	+	+	4	<0.5	39
−	−	−	−	−	+	77	82	—
−	−	−	+	+	+	5	<0.5	—

+ = lysis; − = no lysis. Phages were as described under Materials and Methods. H5gal indicates that the test was performed in the presence of 0.2% of glucose and galactose. CM-resistant clones were passed through single-cell reisolation five times at 42°. Most clones lost the CM marker.

ciencies (see Ornellas and Stocker, 1974, for details). Other P1 stocks (P1kc, P1vir) behaved as P1CM *clr-100* indicating that adsorption is the main barrier to P1 infection in *S. typhimurium*.

Strains with mutations in the *galE* gene were found to be P1 and C21 sensitive, P22 and F0 resistant in medium lacking galactose. In the presence of galactose (and glucose, to prevent the galactose-killing-effect), phage sensitivities were reversed to different extents depending on the leakiness of the *galE* mutation. These results are in agreement with those of Ornellas and Stocker (1974).

2. Generation of S. typhimurium P1-sensitive Mutants. S. typhimurium P1-sensitive mutants were recovered by the following methods: a) P22-mediated transduction of *hut*$^+$ *galE503* with SL3684 as donor and *S. typhimurium* strains unable to utilize histidine as nitrogen source (Gutnick *et al.*, 1969) as recipients. The two markers are about 50% cotransducible (Sanderson, 1972), such that roughly 50% *hut*$^+$ transductants should carry the *galE503* allele. In actual practice, the cotransduction frequencies ranged from less than 0.4% to 58%. Moreover some recipients failed consistently to be transduced to *hut*$^+$. The explanation for this, phenomenon is not known. b) Screening for resistance to phage F0 (Jan Neuhard personal communication). c) Screening for resistance to P22 and d) Direct selection for resistance to high levels (12.5 μg/ml) of chloramphenicol mediated by P1CM *clr-100*.

As shown in Table 2, 100% of chloramphenicol-resistant clones are P1-sensitive and 16% are *galE* (line 1), about 18% of F0-resistant clones are P1-sensitive and 8% are *gal E*; about 18% of P22-resistant clones are P1-sensitive and 4% are *gal E*. The results shown were obtained with the wild type LT2 strain, other strains gave different absolute numbers. Supplementing plates with 0.5% glucose tended to increase the yield of *galE* by as much as two-fold. Results shown in Table 2 are in agreement with the detailed analysis of Ornellas and Stocker (1974).

3. Behavior of P1CM clr-100 Salmonella Lysogens. The P1 stock used has a thermo-inducible repressor and the CM marker that makes lysogens reistant to

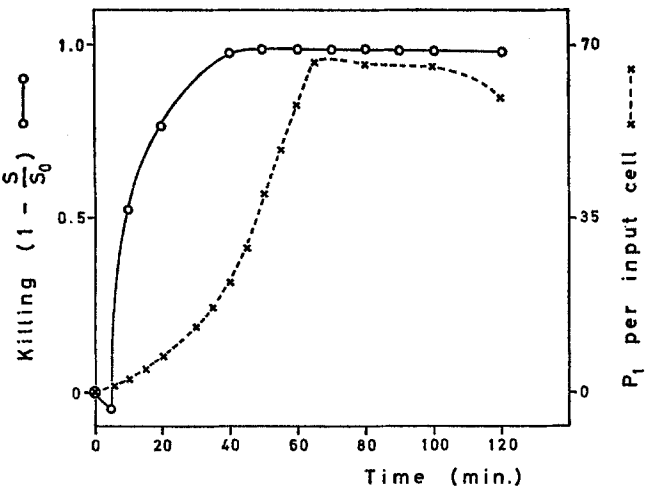

Fig. 1. Kinetics of P1 induction at 42°. MA82 was grown ON at 30° in LB Broth and diluted to about 10^5 cells/ml. Aliquotes (0.5 ml) were dispensed to a series of tubes containing 4.5 ml LB Broth prewarmed at 42°. At 5 min intervals one tube was taken and immediately plated to determine survival (o–o–o). The rest of the tube was re-incubated at 37° for additional 180 min, sterilized with $CHCl_3$ and assayed for phage (×–×–×)

chloramphenicol at low temperature. The phage strain is thus suitable for rapid, positive selection of lysogens and for studying events following induction. Lysogens were readily recovered in *galE* strains (and other strains previously made P1-sensitive by the methods outlined above) by mixing cells with phage and plating the mixture on LACM plates which were then incubated at 30° for about 48 h. P1CM *clr-100* lysogens released P1CM *clr-100* and as a general rule failed to grow at 42°. Some presumptive lysogens that released phage and grew at 42° were deemed to be revertants of the *clr-100* marker and were discarded.

Salmonella—P1CM *clr-100* lysogens like *E. coli*—P1CM *clr-100* lysogens (Rosner 1972) were killed at temperatures above 35° with efficiencies that depended on the temperature; e.g. at 35° about 50% of the cells were killed in 45 min while at 41° nearly 100% of the cells were killed. Lysogens were stable at temperatures up to 33°.

Results shown in Fig. 1 indicated that at 42°, killing of lysogens was very rapid after the first five min. and it reached a plateau after about 40 min. Further incubation at the nonpermissive temperature did not result in an increase in killing. Killing at 40° and 41° followed essentially the same kinetics as for 42°. Killing at lower temperatures (e.g. 37°) followed slightly different kinetics, however.

The effect of temperature on P1 release was also measured. A culture of MA82 was "induced" at several temperatures for 45 min. and cultures were reincubated at 30 for further 180 min. to allow for phage development. In all cases, P1 released per input cell was less than 1.0. These results indicated that P1CM *clr-100* failed to develop in *S. typhimurium* at 30°. This interpretation was substantiated by the

Table 3. Adsorption of P1CM *clr-100* to SL3684

m.o.i.	Unadsorbed P1 (%)	Cell survival (%)	CM-resistant colonies (%)
10	63.6	0.018	99.3
5	25.1	0.04	95.3
2	4.2	0.92	47.0
1	3.5	6.7	12.6
0.5	0.040	7.0	—
10^{-1}	0.010	96.0	—
10^{-2}	0.015	99.9	—
10^{-3}	0.017	—[a]	—
10^{-4}	0.001	—	—
10^{-5}	0	—	—

Four ml of ON cells were mixed with 1.0 ml of appropriate phage dilution and incubated at 37° for 15 min. Mixture was centrifuged and supernatant titered on MA73 (col.2). Pellet was washed twice with phosphate buffer (pH 7.0) and dilutions were plated on nutrient plates that were incubated at 42° and scored 24 h later (col.3); and on LACM plates were incubated at 30 and scored after 48 h (col.4).
[a] Not determined.

observation that after overnight incubation, all P1 stocks tested failed to make plaques on *S. typhimurium* strains at 30°, while plaques appeared at 37°.

When "induced" cultures were reincubated at 37° rather than at 30°, to allow for phage development, mature P1 particles were recovered at frequencies comparable to (although lower than) those reported for similar *E. coli* lysogens (Rosner, 1972). Fig. 1 shows the kinetics of P1 release after induction of MA82 at 42° and reincubation at 37°.

The highest P1 titers were obtained by inducing lysogens at 40° for 55 min followed by 110 min at 37° with vigorous shaking. It should be pointed out that some lysogens could not be induced efficiently, in those cases, it was more convenient to prepare P1 by lytic infection.

4. Adsorption of P1CM clr-100 to gal E Mutants. In using P1 in a new species, it is important to determine the parameters affecting adsorption. This is important not only in the preparation of high titer lysates and for transduction but also for metabolic studies.

Results of adsorption experiments are given in Table 3 which indicated that in 15 min. nearly 100% P1 particles had adsorbed, if the m.o.i. was about 1.0 or less; with higher m.o.i.'s the frequency of unadsorbed P1 increased considerably. Other experiments (results not shown) indicated that no more adsorption took place after prolonged incubation and that P1 was able to absorb normally in the absence of $CaCl_2$.

Surviving cells and CM-resistant clones were measured as indicators of phage adsorption, although the results were consistent (Table 3), as a quantitative parameter of adsorption, they were not useful.

5. Lytic Infection. Since some lysogens could not be induced to yield high titers, and because it seems important to test induced lysates, as well as lytic-infection lysates, for their ability to mobilize genetic markers, optimum conditions

for growth of P1 by lytic infection were determined, and found to be as follows: 0.2 ml of host cells grown overnight at 37° without shaking, were mixed with 1–3 × 10^6 p.f.u. of P1 (0.1 ml), the mixture was incubated for 15 min. at 40° and then overlaid in LSA, on LA plates. Plates were incubated at 40–41° for 8–10 h; at this time, 5.0 ml volumes of LB were pipeted in per plate, and plates kept at 4° for at least 10 h. The LSA layer was then finely triturated with a glass rod, centrifuged for 20 min. at 5000 rpm and stored over chloroform at 4°.

Phage lysates prepared this way usually contained about 10^{10} p.f.u./ml. Drastic variations in the procedure resulted in lower phage titers. Most P1 lysates used in transduction were prepared by this methods since it appeared to be the most convenient one.

Titers dropped remarkably (by as much as a factor of 10^3 in one year) after prolonged storage at 4° and much faster at room temperature. Mixing in a vortex-mixer also resulted in reduced viabilities. For these reasons, P1 stocks were renewed periodically.

6. Transduction. The aim of this study was to establish a system to readily utilize P1 as transduction vector in *S. typhimurium*. Hence, parameters affecting transduction were measured.

The mode of P1 preparation, that is whether P1 was prepared by lysogen induction or by lytic infection did not affect the frequency of recovery of several markers tested, representing scattered regions on the *S. typhimurium* chromosome. These results are in agreement with those reported for *E. coli* (Rosner, 1972). One hundred *his203*$^+$ transductants were tested and found to be due to complete transduction, involving chromosomal integration and not from lysogenization with a special transducing derivative.

Transductant yields were the same in the presence and absence of $CaCl_2$. All mapping crosses were performed without $CaCl_2$.

Incubation of transduction plates at 30° resulted in 2-fold higher transductant frequentcy with respect to 37° and about 10-fold higher with respect to 42°. On the other hand, about 50% of transductants recovered at 30° were P1-lysogenic (CM-resistant, temperature sensitive and released P1 spontaneously), while none (less than 1%) of the transductants recovered at 37° or 42° was P1-lysogenic. Incubation of transduction plates at 37° was adequate for mapping purposes, when P1-lysogenization was not desirable. There is the added advantage that at 37° (and at 42°), transductants can be scored after 16 h incubation while at 30° transductants are visible only after 36 h.

The effect of m.o.i. on transductant yield was tested. Results shown in Table 4, for m.o.i.'s ranging from 10^{-2} to 10, indicate that there was little difference in the efficiency of transduction (transductant per input phage unit), for m.o.i. between 0.1 and 1, although the absolute number of transductants varied accordingly. For m.o.i. between 2 and 5 the efficiency of transduction decreases slightly and at m.o.i. 10, there is a 10-fold difference as compared to m.o.i. of 1. The lower efficiencies of transduction were probably consequences of unadsorbed phage and of certain amount of killing. For mapping purposes, multiplicities higher than 0.5 were adequate, since the absolute number of transductants does not greatly vary.

7. The trp-hemA Region. P1-mediated transduction data for the *trp-hemA* region are summarized in Fig. 2. All P22-linked genes were also P1-linked; in

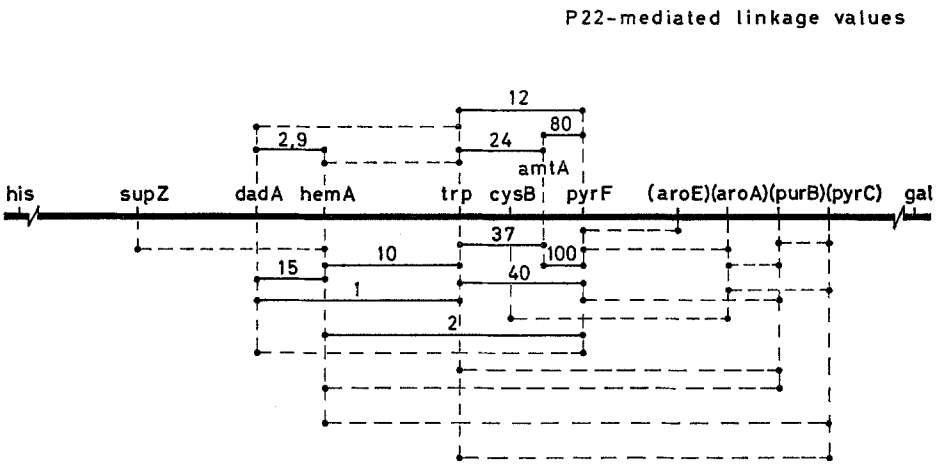

Fig. 2. Transduction linkage for the *trp* region of the *Salmonella* chromosome. Not drawn to scale. The lower part of the line shows P1-mediated linkage values. A broken line joining any two given markers indicates that no linkage was detected (less than 0.5%). The upper part of the line shows published P22-mediated linkage values as follows: unlinked markers (Sanderson, 1972), *hemA–dadA* (Wild *et al.*, 1974), *trp–pyrF*, *trp–amtA*, *pyrF–amtA* (Wiater and Klopotowski, 1972)

Table 4. Multiplicity dependence of P1-mediated transduction in *S. typhimurium*

m.o.i.	*his*$^+$ transductants per input p.f.u.
10	1.25×10^{-7}
5	2.66×10^{-7}
2	4.65×10^{-7}
1	1.45×10^{-6}
0.5	2.20×10^{-6}
10^{-1}	1.20×10^{-6}
10^{-2}	2.10×10^{-7}

Recipient was MA14 (*his203*) and donor SL3684. 0.5 ml of ON recipient was mixed with 0.5 ml vector phage, after 15 min at 37°, 0.25 ml were spread per plate. m.o.i.'s were calculated assuming 3.0×10^9 cells/ml and were not corrected for unadsorbed phage. Fifures shown were obtained in a single experiment with two plates. Other experiments gave similar results.

addition, previously undetected linkage relationships, i.e. *trp-hemA*, *trp-dadA*, were detected with P1. The relative order of genes on the right side of *pyrF* (i.e. *aroE*, *aroA*, *purB* and *pyrG*) could not be determined, since no cotransduction was detected between any two markers tested.

8. Order of hemA-trpA-pyrF. Preliminary transduction tests showed *trp* and *pyrF* to be approximetaly 10% and 2% contransducible with *hemA* respectively (Fig. 2). P1 phage propagated on *trpA* 52, *pyrF 146* (MA45) was used as donor with *hemA 205* (MA22) as the recipient in a threepoint transduction test. Fig. 3 depicts

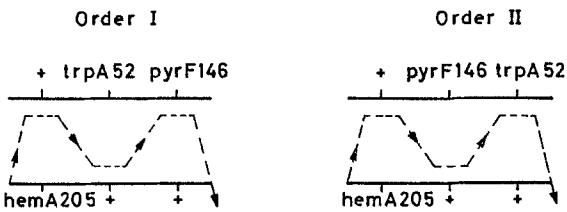

Fig. 3. Two alternative possibilities for the gene order of the *hemA–trp–pyrF* region: *trpA52 pyrF146* donor × *hemA205* recipient

Table 5. Three-point transduction cross[a] in the *trp-hemA* chromosomal region

Genotype of recombinants			Number of recombinants	Frequency (%)
hemA	*trpA*	*pyrF*		
+	52	+	42	8
+	+	146	0	0
+	52	146	5	1
+	+	+	473	91
			520	100

[a] Recipient: MA22 (*hemA205 his 3501 ilvA5 galE542 hut*$^+$). Donor: MA45 (*trpA52 purF146 galE*). Selection for *hemA*$^+$ on B plates.

two possible gene orders. If gene order I is correct, the minority recombinant class should have the *trpA 52*$^+$ *pyrF146* phenotype; if order II is correct then the minority recombinant class should have the *trpA52 pyrF146*$^+$ phenotype. In fact, *trpA52 pyrF146*$^+$ recombinants appeared with a frequency of 8% (42/520) (Table 5). Thus, the more favored gene other of this region in order I: *hemA-trp-pyrF*.

Since *hemA-dadA* were 15% contransducible and *trp-dadA* were only 1% and *pyrF-dadA* were only 1% and *pyrF-dadA* were less than 0.5% contransducible (Fig. 2), it follows that the order of genes in that region was *dadA-hemA-trp-pyrF*.

9. The purC-purF Region. Linkage data for the *purC-purF* region are summarized in Fig. 4. Most of the data presented had not previously been detected by P22- mediated transduction (Sanderson, 1972).

A few apparent peculiarities and inconsistencies should be commented upon.

The *trzA* gene is shown to the right of *cysA*, distal to *purC* despite that *purC-trzA* linkage (3.4%) was higher than *purC-cysA* linkage (1.0%), because P22-mediated transduction (Hulanicka and Klopotowski, 1972) placed *trzA* next to *cysA* and deletion mapping (Cordaro and Roseman, 1972) placed *trzA*, unambiguously, between *cysA* and *pts*, as shown in Fig. 4. The inconsistencies in linkage values could conceivably be due to high mutation rate of recipient to *trz*.

Linkage between *purF* and *dhuA* varies slightly with genetic background (32–56%), despite that the same, *dhuA1*, allele was used as donor in three different cases.

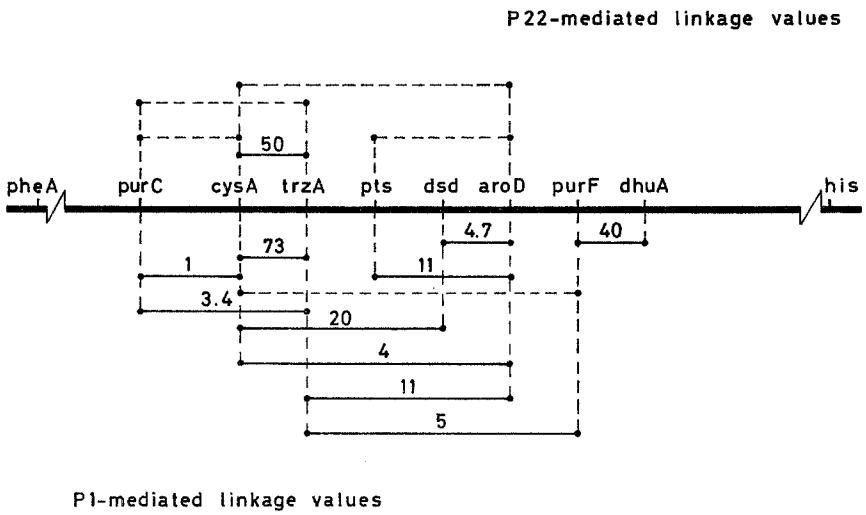

Fig. 4. Transduction linkage for the *cysA* region of the *Salmonella* chromosome. Not drawn to scale. A broken line indicates that no linkage (less than 0.5%) was detected between any two given markers, The lower part shows P1-mediated linkage Values. The upper part of the line shows published P22-mediated linkage values as follows: unlinked markers (Sanderson, 1972), *cysA–trzA* (Hulanicka and Klopotowski, 1972)

The *dsd* gene has been tentatively placed between *cysA* and *aroD*. The data presented were not reliable, however. A laborious analysis of recombinants with *cysA20* (MA34) and *aroD166* (MA16) as recipients and *dsd-2* (MA12) as donor, revealed some unusual pecularity of that chromosomal region. One *cysA20*$^+$ *dsd-2* (out of 20%) was streaked for single cell on an E plate. Ten single colonies were picked up and tested for DL-serine sensitivity. Only 2 (20%) were found to be sensitive (*dsd-2*), while 8 (80%) were found to be resistant (*dsd-2*$^+$). Again, ten single colonies were tested; 2 (20%) were *dsd-2* and 8 (80%) were *dsd-2*$^+$. One of the eight *dsd-2*$^+$ colonies was, in turn, streaked for single cell. Ten colonies were tested; 3 (30%) were found to be *dsd-2* (sensitive) and 7 (70%) *dsd-2*$^+$ (resistant).

One hundred and six (106) *aroD166*$^+$ recombinants were tested for DL-serine sensitivity, 23 (22%) were found to be *dsd-2* (sensitive). When retested, only 6 (5% of total) remained *dsd-2*. It was clear, however, that *dsd-2* was linked to both *cysA* and *aroD* and most likely in between them, since, were it on either side, linkage values, as unreliable as they were, would be much higher with the closer marker.

Discussion

S. typhimurium is outside the host range of coliphage P1 primarily because of failure to adsorb; in addition, P1 is sensitive to the *S. typhimurium* host specificity systems (J. Gerits, personal communication). Once the adsorption barrier is removed by one step mutations in some cell-wall genes of *S. typhimurium* (Ornellas and Stocker, 1974), P1 is able to develop in *S. typhimurium*. This is in contrast to the observation that P1 adsorbs and kills *Pseudomonas aeruginosa* and *Serratia*

marcescens, but fails to produce mature P1 particles (Amati, 1962 quoted in Holloway, 1969).

It also differs from the behavior of Lambda phage in *S. typhosa* and *Shigella flexneri* (Kayajanian, 1970; Gemski *et al.*, 1972), in which additional host or phage functions are required for phage development.

It seems that *Salmonella* strains with mutations in the *galE* gene are the most useful for using P1, because of the possibility to reverse the rough phenotype in the presence of galactose, such strains regain P22 sensitivity in the presence of galactose (and glucose, to circumvent the galactose killing effect). In terms of genetic analysis, this might be very important. Moreover *galE* mutants can be generated with relative facility, by a variety of positive-selection procedures, of which only a few have been explored.

Behavior of coliphage P1 in *S. typhimurium* strains with mutations in the *galE*gene, closely resemble the bahavior of the phage in its "natural" host, *E. coli* (Scott, 1968; Rosner; 1972). In *S. typhimurium*, P1 does not require $CaCl_2$ for adsorption as it does in *E. coli* (Franklin, 1969). The P1 adsorption site on the cell-wall of *galE* strains is different enough from its *E. coli* counterpart to not require $CaCl_2$. The P1 *S. typhimurium* (*galE*) relationship is somewhat similar to the relationship of *E. coli* with some P1 strains described by Franklin (1969)

Coliphage P1 does not develop to maturity in *S. typhimurium* at 30°, although it does in *E. coli*. Presumably, at 30°, phage repression is very effective. Moreover, lysogenization of *S. typhimurium* by P1 seems to differ from lysogenization of *E. coli* (Rosner, 1972) and this problem is under study.

Phage PICM *clr-100* readily mobilizes prototropic markers from *S. typhimurium* donors to *S. typhimurium* recipients that had been made P1-sensitive by the methods outlined under Results. Most of the strains used were *galE* mutants, but other, uncharacterized rough strains, were also effective.

Transduction by P1 in *S. typhimurium* closely transduction by P1 in *E. coli* resembles (Lennox, 1955; Rosner, 1972), except that $CaCl_2$ is not required for adsorption *S. typhimurium*.

P1 transducing fragments carrying a given *S. typhimurium* marker are heterogeneous, since, some sequentially linked genes are not simultaneously cotransducible. For example *dadA* is linked to *trp* and *trp* is linked to *pyrF*, but *dadA* is not linked to *pyrF* (Fig. 2).

The *trp* region encompasses a well-known inversion between *E. coli* and *S. typhimurium* (Sanderson and Hall, 1970) shown in Fig. 5. Data shown in Fig. 2 and Table 5 indicates that the relative order of genes is:

his ... dadA–hemA–trp–pyrE$^-$ *... pyrC.*

Thus, to date, the leftmost genes within the inversion are *dadA* in *S. typhimurium* and *tyrR* in *E. coli* (Taylor and Trottner, 1972) and the rightmost genes are *tyrR* in *S. typhimurium* (Sanderson, 1972) and *dadR* (Kuhn and Somerville, 1971) in *E. coli* (Taylor and Trottner, 1972).

The *purB* gene could conceivably be included in the inversion, since *pyrC-purB* cotransduction frequency is 3.5% in *E. coli* (Cronan *et al.*, 1972), and undetectable in *S. typhimurium* (Fig. 2). Alternatively a second inversion, including at least, *aroA*, *purB* and *purC* could be present.

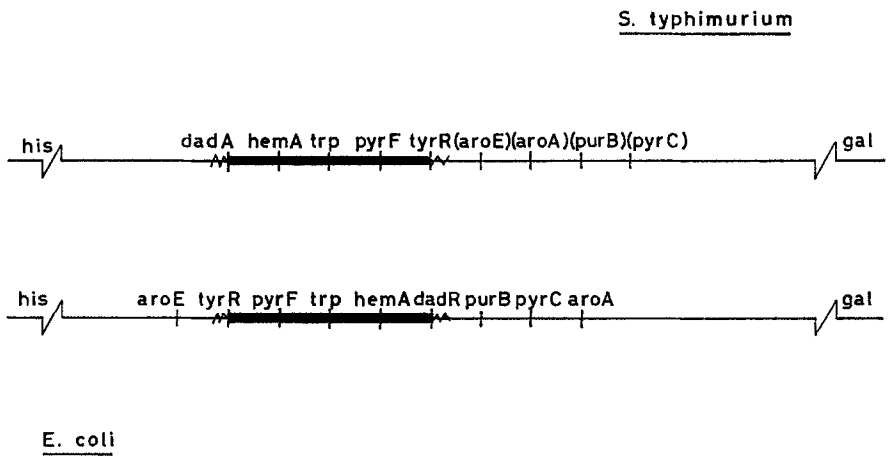

Fig. 5. A comparison of the *trp* region of *S. typhimurium* and *E. coli*. The figure was drawn such that the *trp* operons coincide and it was not drawn to scale. The thick lines demark the known limits of the inversion. The *E. Coli* gene order vas taken from Taylor and Trotter (1972) and the gene order of *S. typhimurium* was taken from Sanderson (1972), except for *hemA* and *dadA* that were taken from Fig. 2. Nomenclature is that of *S. typhimurium*, thus *aroE* is *aroD* in *E. coli*

Closely linked genes, by conjugation, in the *cysA* region, not linked by P22 transduction, are linked by P1.

The position of *dsd* could not be accurately determined because of segregation of donor and recipient phenotypes at high frequency, which is probably due to chromosomal duplications of the type reported by Hill *et al.*, 1969. Clearly, a more detailed analysis of the region is desirable, since peculiar events appear to take place (Cordaro and Roseman, 1972; Hulanicka and Klopotowski, 1972).

The size of the P1-*Salmonella* transducing fragment was not determined. In all cases tested, P1 was more efficient, in cotransduction than P22. This is to be expected since the DNA content of a P1 particle is about 60 million daltons (Ikeda and Tomizawa, 1968) while that of P22 is about 23 million daltons (Thomas and Machattie, 1967). Thus the P1 DNA molecules is more than twice the length of P22.

Recently, an equation that correlates cotransduction frequencies to physical distance (in average gene lengths) between two markers, has been published (Kemper, 1974). A comparison of physical distances between markers as revealed by P1 and P22 transduction was made (Table 6).

In general, there is good agreement between the two phages, although there is some indication that, in at least some cases, factors other than physical distance might have bearance on the cotransduction frequency.

The P1-mediated transduction system described here should, expectably, produce more accurate and reliable mapping parameters in *S. typhimurium*. It is expected that eventually, most genes will be found sequentially linked as it is the case in *E. coli* (Taylor and Trotter, 1972). The transduction system should also be useful in the construction of strains: e.g. should a particular *trp* allele be required

Table 6. A comparison of linkage relations in *S. typhimurium* obtained by P1- and P22-mediated transduction

Linked genes	P1		P22	
	a	b	a	b
hemA–dadA	15	45	2.9	35
trp-dadA	1	81	0	>40
trp–hemA	10	52	0	>40
trp–pyrF	40	23	12	23
trp-amtA	37	24	24	16
pyrF–amtA	100	0	80	5
cysA–purC	1	81	0	>40
cysA–trzA	72	7	50	7
cysA–aroD	4	65	0	>40
pts–aroD	11	50	0	>40

a = Contransduction frequencies as presented in Fig. 2 and 4. b = Physical distances given in gene lengths, calculated as in Kemper (1974). Maximum P1 length was assumed to be 88 genes and maximum P22 length was assumed to be 40 genes.

in a strain with defined genetic markers, the *trp* allele can be transferred to the strain after positively selecting for *hemA* mutants (Sasarman *et al.*, 1969) and collecting *hemA*$^+$ *trp* cotransductants.

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