Bacterial Donor Mutants Affecting the Efficiency of Generalized Transduction by Salmonella Phage P22

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(Received 21 March 1978)

P22 lysates of some mutants (LD mutants) of *Salmonella typhimurium* gave lower transduction frequencies than phages grown on the wild type. The lower frequency was not due to the production of reduced numbers of transducing particles, or superinfection exclusion, or decreased incidence of integration. The defect was in a host-controlled modification-restriction system. LD mutants possess $m^{-}r^{-}$ characteristics. The degree of expression of transducing particles varied with the phenotype selected, although some gene products, including the numbers of plaque-forming phages of P22, were not affected. However, several other *Salmonella* phages, some of them related to P22, exhibited high sensitivities. The LD system appeared to be identical to Colson's SA system (Colson & van Pel, 1974).

INTRODUCTION

During a study of generalized transduction mediated by *Salmonella* phage P22, we isolated phage mutants with increased or decreased transduction abilities (HT and NT mutants; Schmieger, 1972). Mapping of some of these mutants revealed that there are at least five P22 genes influencing the production of transducing particles (Raj *et al.*, 1974). To elucidate further the processes leading to the production of transducing particles, we have examined the possible involvement of host functions. These were implicated by the isolation of bacterial mutants (LD mutants) whose P22 lysates gave decreased frequencies of transduction. Results described here indicate that this effect is due not to the production of reduced numbers of transducing particles, but to damage to a modification-restriction system which only affects transducing DNA.

METHODS

Bacterial and phage strains are listed in Tables 1 and 2.

Abbreviations and notations. m.o.i., multiplicity of infection, i.e. the number of phages to one cell; e.o.p., efficiency of plating; p.f.u., plaque-forming units (approximately identical with infective phage); HMR, host-controlled modification-restriction system. LD indicates bacterial mutants exhibiting the LD phenotype described in this paper; LD⁺ indicates strains which do not exhibit the LD phenotype and are wild type with respect to the relevant genes. The bacterial strain on which a phage lysate has been propagated is indicated by the following notation: H5:LT2 means phage H5 grown on strain LT2, etc.

Media. Nutrient broth (NB) contained 8 g Bacto nutrient broth and 5 g NaCl in 1 litre deionized water. For nutrient agar (NA), NB was solidified with 1.5% (w/v) Bacto-agar. M9 medium contained 15 g Na₂HPO₄·12H₂O, 3 g KH₂PO₄ and 0.5 g NaCl in 1 litre deionized water; after sterilization, 2 ml glucose (20%, w/v) and 1 ml MgSO₄ (0.1 M) were added. For M9 agar, M9 medium was solidified with 1.5% (w/v) Bacto-agar. TCG medium, prepared according to Kozinski & Szybalski (1959), was used for labelling bacterial and phage DNA with either [³H]thymidine (20 µCi ml⁻¹; 5 Ci mmol⁻¹) or [¹⁴C]thymidine (0.5 µCi ml⁻¹; 50 mCi mmol⁻¹), both in the presence of 1 µg unlabelled thymidine ml⁻¹ and 250 µg deoxyadenosine ml⁻¹.

Labelling of phage DNA with ³H or ¹⁴C. A thymine-requiring strain, e.g. T4/2 or LD1.1, was grown in

Table 1. *Salmonella typhimurium* strains

Strain designation	Description	Source
LT2	Prototrophic, LD ⁺⁺	M. Demerec
HisB22	<i>hisB22</i>	M. Demerec
T4/2	<i>thy-2</i>	This laboratory
LD10	Prototrophic, LD*	This paper
LDx.y	<i>thy-2</i> , LD*, x and y represent different serial numbers	This paper
LD1.1 <i>thy</i> ⁺	Prototrophic revertant from LD1.1	This paper
SA1145	<i>proAB391, metA22, trpB2, hisF1009, ilvA99, xyl-1, strA201</i>	K. E. Sanderson
SA607	F ⁺ , <i>serA15</i>	K. E. Sanderson
SA1134	<i>phe-47, metA22, trpB2, hisF1009, xyl-1, strA201</i>	K. E. Sanderson
SA624	<i>cysE1709, pyrE125, xyl-3</i>	K. E. Sanderson
SA4	<i>leuA124, araB9</i>	K. E. Sanderson
SU687	<i>trpA52, cysB12, pyrF146</i>	K. E. Sanderson
SU418	HfrB2, <i>proA26</i> , (P22)	K. E. Sanderson
SB109	<i>metE338, ilvC401, araB9, strA149</i>	P. E. Hartman
SH480	<i>pyrB96</i>	P. H. Mäkelä
4423	<i>proC90, hsdSA22 (m^{-r-}), str</i>	C. Colson
4420	<i>metA22, trpB2, xyl-1, strA201, hsdSA (m^{-r-})</i>	C. Colson

* LD, Phenotype of donor strains which exhibit reduced frequencies of transduction.

Strains used as recipients in transductions were used in lysogenic form and marked by the symbol (P22).

Table 2. *Phage strains*

Strain designation	Description	Source
P22 strains		
P22	Wild type	This laboratory
H5	<i>c2</i>	This laboratory
HT13/4	<i>c2</i> , high transducing mutant	This laboratory
Other Salmonella phages		
FO		B. A. D. Stocker
9NA		B. A. D. Stocker
A1a		B. A. D. Stocker
A3		B. A. D. Stocker
ES18h1		B. A. D. Stocker
L		B. A. D. Stocker
KB		B. A. D. Stocker
Px1c2	Hybrid between P22 and Py, <i>c2</i>	H. H. Prell

TCG medium containing 20 µg thymidine ml⁻¹ to a density of 2 × 10⁸ cells ml⁻¹. Bacteria were then centrifuged, resuspended in the appropriate labelling medium (see above) and infected with phages at an m.o.i. of 10. After 90 min shaking at 37 °C, chloroform was added and incubation was continued for a further 10 min. Debris was removed by centrifugation. Radioactive phages were purified by centrifugation in a stepwise CsCl gradient as described by Schmieger (1972).

Differential labelling of DNA of transducing and plaque-forming phages. This was performed as described by Schmieger (1972) except that 20 µCi [³H]thymidine and 5 µCi ³²P were used.

Re-extraction of radioactive DNA from infected cells. An equal volume of lysis mixture [0.1 M-Tris/HCl (pH 8.0), 0.1 M-EDTA (pH 8.5), 0.01 M-NaCN and 100 µg lysozyme ml⁻¹; Botstein, 1968] was added to the bacterial suspension and incubated for 10 min at 37 °C. Sarcosyl NL 30 (Ciba-Geigy) was then added (final concentration 1 %, v/v) and incubation was continued for 20 min at 65 °C. DNA was extracted from this crude lysate by the phenol method (Schmieger, 1971).

Sucrose gradients. Neutral sucrose solutions (5 and 20 %, w/v) were prepared according to Botstein (1968) in 0.02 M-Tris/HCl (pH 8.0), 0.01 M-EDTA (pH 8.5) and 0.3 % (v/v) Sarcosyl NL 30. Gradients were mixed from 2.2 ml 5 % solution and 2.1 ml 20 % solution and layered over a cushion of 0.5 ml CsCl solution (= 1.8 g ml⁻¹). A 0.1 ml portion of the [³H]DNA to be assayed and 0.1 ml of [¹⁴C]DNA as a reference were layered on top. Centrifugation was carried out in an SW 50.1-rotor in a Spinco L2 ultracentrifuge at 35000 rev. min⁻¹ at 23 °C for 3 h. Fractions of 10 drops were collected and prepared for scintillation counting as described by Schmieger (1970).

Fig. 1. Strains LT2 (= LD⁺) and LD were plated in appropriate dilution on NA and incubated overnight (a). The colonies were replica-plated on to M9 agar seeded with 10⁷ p.f.u. of phage H5. After 6 h incubation, cells were killed by chloroform vapour. A concentrated overnight culture of strain HisB22(P22) was then sprayed over the plates as transduction recipients. Transductants appeared after another 2 d incubation only at the positions of LT2 colonies (b). [The two colonies (top right and top centre) which appeared after incubation of the replica plates are contaminants.]

Mutagen treatment of bacteria. Cultures were grown with aeration at 37 °C in M9 medium (if necessary supplemented with the growth factors required) to a titre of 5×10^7 cells ml⁻¹. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) was added to a final concentration of 4 µg ml⁻¹, and incubation with aeration was continued for 30 min. Bacteria were then centrifuged, washed with buffer, resuspended in an equal volume of prewarmed NB and again incubated with aeration for 30 min at 37 °C before plating on NA in appropriate dilution. Further manipulations depended on the properties of the mutants sought.

Isolation of LD mutants. LD mutants (Low Donor ability, since they were believed initially to affect the production of transducing particles) were isolated by a modification of the method used by Schmieger (1971) for isolating phage mutants with altered transducing ability. Cultures of *S. typhimurium* strains LT2 (prototrophic) or T4/2 (*thy*) were treated with NG as described above. To eliminate unwanted auxotrophic mutants (about 18 to 20 % of the surviving bacteria), the treated culture was plated on M9 agar, or for strain T4/2, on M9 agar supplemented with 20 µg thymidine ml⁻¹ (M9/20), to give about 100 colonies/plate, and the plates were incubated at 37 °C. These colonies were replica-plated on to M9 or M9/20 plates which had been seeded with about 10⁷ H5 phage particles, and incubated for 6 h at 37 °C. During this time localized lysates were expected to develop at the positions of the colony prints after infection of the cells with H5 on the plates. The surviving cells were killed by pouring chloroform into the top lids of the plates and incubating for 30 min at 37 °C. The top lids were then removed and incubation was continued for 1 h to evaporate the chloroform. Then a fresh overnight culture of strain HisB22(P22), a recipient for transduction that had been concentrated 5:1 in saline plus 20 % (w/v) NB, was blown on to the plates with a perfume sprayer and the plates were incubated for another 2 d. During this time *his*⁺ colonies appeared at the positions of the printed colonies after infection of recipient cells by *his*⁺ transducing particles present in the localized lysates. By comparison with the master plates, colonies could be found where the replica-plated areas did not show transductants (Fig. 1). These colonies were picked from the master plates, purified by restreaking and subjected to further analysis.

Transductions. Phages were added at an m.o.i. of 10 to a fresh overnight culture of the auxotrophic recipient strain and the mixture was incubated at 37 °C for 10 min. Appropriate dilutions (in NB) were then plated on M9 agar and incubated for 2 d at 37 °C. Transduction frequencies are given as T/P ratios, i.e. the ratio of the number of recombinant transductants to the number of plaque-forming units.

RESULTS

Characterization of LD mutants

The mutants isolated by the procedure described in Methods could be either truly resistant (or insensitive) to P22 and so produce neither p.f.u. nor transducing particles, or their mutations may affect the frequency of transduction (LD mutants). In order to eliminate P22-insensitive mutants we cross-streaked all the mutants isolated against H5:LT2. In a typical experiment, 35 out of 58 colonies without transductants (2.2% of colonies tested by the spraying method) were P22-insensitive, and 23 produced phage lysates with normal numbers of p.f.u. but showed reduced transduction frequencies when assayed for *his*⁺ transduction. The latter were therefore presumed to be LD mutants. The transduction frequencies for the marker *hisB22*⁺ of LD-grown H5 lysates were all significantly lower than those for wild-type phages, although the degree of reduction differed from mutant to mutant (Table 3). For further studies, the following LD mutants were chosen: LD10 (prototrophic), and LD1.1, LD1.2, LD2.1, LD2.2, LD3.1 and LD3.2 (all *thy*). Mutants with smaller reduction effects were not analysed further.

It was of interest to know whether these reductions were specific for *his*⁺. We therefore determined the transduction frequencies for several markers distributed widely around the chromosome of *S. typhimurium* (Sanderson, 1972). It was known that some of these markers show very low T/P ratios for phage H5 (Schmieger, 1972), and so further reduction due to the LD effect might not be demonstrable. Transductions were therefore carried out with a mutant of H5, HT13/4, which produces a higher number of transducing particles than the parental strain. Mutant LD10 was chosen as LD donor and wild-type LT2 served as an LD⁺ control. The transduction frequencies of *trpB2* and *ilvA99* showed similar reductions (by a factor of about 100) to *hisB22*. Transduction of other markers, notably *pyrF146* and *leuA124*, was reduced only by a factor of 10, and transduction frequencies of *metE338*, *cysE* and *phe-47* were not affected by the LD phenomenon (Table 4).

Differential labelling of transducing and plaque-forming phages

Differential labelling of the DNA of p.f.u. and transducing particles was based on the fact that transducing phages contain mainly bacterial DNA synthesized before infection, whereas almost all p.f.u. contain newly synthesized DNA (Schmieger, 1968). The DNA of phages was labelled with ³²P and that of transducing particles with ³H. The ³H/³²P ratios for LD lysates and the T4/2 control were similar (Table 5). Thus in LD lysates, the ratios of transducing particles to p.f.u. were similar to those in lysates grown on wild-type strains (with respect to LD). Since the titres of p.f.u. in comparable lysates were similar (Table 5), the absolute numbers of transducing particles must also be similar. Therefore, the decreased T/P ratios of LD-grown lysates (Table 3) cannot be explained by the formation of smaller numbers of transducing particles.

What causes the LD phenotype?

At least four effects could account for the reduced numbers of transductants yielded by LD mutants. (a) LD-grown lysates adsorb to LD⁺ strains with reduced efficiency. (b) Transducing DNA fragments from LD donors integrate less frequently in LD⁺ recipients. (c) Bacterial DNA of LD strains is more sensitive in lysogenic recipients to the super-infection exclusion systems of P22 prophage than is DNA from wild type LD⁺. (d) LD strains are damaged in a modification-restriction (HMR) system which causes degradation of transducing DNA in restrictive LD⁺ recipients.

Hypothesis (a) was excluded by showing that LD-grown phages plate on LD and wild-type LD⁺ indicators with almost identical e.o.p. (Table 6). Transducing particles differ from plaque-forming phages only in their DNA; they have the same protein shell and base

Table 3. *Transduction frequencies from some LD mutants for the marker hisB22⁺*

Lysate	T/P ratio* for <i>his</i> ⁺ transduction	Relative transduction frequency† (%)
H5:LT2	1.3×10^{-6}	100.0
H5:LD10	4.5×10^{-8}	3.4
H5:LD1.1	1.7×10^{-9}	0.13
H5:LD1.2	3.7×10^{-8}	2.9
H5:LD2.1	3.0×10^{-8}	2.3
H5:LD2.2	1.5×10^{-8}	1.2
H5:LD3.1	8.2×10^{-8}	6.3
H5:LD3.2	4.5×10^{-8}	3.4
H5:LD3/5	2.1×10^{-7}	16.2
H5:LD9/4	3.3×10^{-7}	23.7
H5:LD5/3	3.4×10^{-7}	24.0

* No. of recombinant transductants/no. of plaque-forming units.

† Expressed as a percentage of the transduction frequency for *hisB22⁺* from H5:LT2 (1.3×10^{-6}).Table 4. *Marker dependence of the LD effect*

Marker selected*	T/P ratio† of lysate		$\frac{T/P_{LT(=LD^+)}}{T/P_{LD}}$
	HT13/4:LT2	HT13/4:LD10	
<i>ilvA99</i> (SA1145)	1.4×10^{-5}	1.6×10^{-7}	87.8
<i>trpB2</i> (SA1145)	3.8×10^{-6}	6.0×10^{-8}	63.3
<i>hisB22</i> (HisB22)	1.3×10^{-5}	3.0×10^{-7}	43.3
<i>proA26</i> (SU418)	3.8×10^{-6}	2.1×10^{-7}	18.1
<i>hisF1009</i> (SA1145)	3.8×10^{-6}	2.5×10^{-7}	15.2
<i>leuA124</i> (SA4)	1.6×10^{-5}	1.3×10^{-6}	12.3
<i>ilvC401</i> (SB109)	1.2×10^{-5}	1.0×10^{-6}	12.0
<i>serA15</i> (SA607)	3.2×10^{-6}	2.7×10^{-7}	11.9
<i>thy-2</i> (T4/2)	1.8×10^{-5}	1.6×10^{-6}	11.3
<i>pyrF146</i> (SU687)	2.6×10^{-6}	2.7×10^{-7}	9.6
<i>araB9</i> (SA4)	1.5×10^{-5}	1.6×10^{-6}	9.4
<i>metA22</i> (SA1145)	9.9×10^{-8}	1.5×10^{-8}	6.6
<i>cysB12</i> (SU687)	1.5×10^{-6}	3.2×10^{-7}	4.7
<i>trpA52</i> (SU687)	4.5×10^{-6}	1.4×10^{-6}	3.2
<i>phe-47</i> (SA1134)	3.0×10^{-7}	1.5×10^{-7}	2.0
<i>cysE1709</i> (SA624)	4.0×10^{-6}	2.2×10^{-6}	1.8
<i>metE338</i> (SB109)	3.9×10^{-6}	4.7×10^{-6}	0.8

* Auxotrophic strains from which markers were selected are indicated in parentheses.

† For definition, see Table 3.

plates. Therefore, reduced adsorption of transducing LD-propagated particles should also be reflected in a reduced e.o.p. of their infective particles.

Hypothesis (b) was also excluded. Bacteria infected by transducing DNA which does not become integrated give rise to abortive transductants (Ozeki, 1956; Stocker, 1956). Therefore, a decreased rate of integration should be reflected by an increased number of abortive transductants. We therefore determined the ratio of abortive/recombinant transductants for *hisB22⁺*. This ratio was usually 13 to 15 for transduction by LT2-grown H5 lysates (Table 6). With LD-grown lysates, the ratio was, at most, increased 2.5-fold (H5:LD2.1), the average for all LD lysates being 15.1, i.e. almost identical to the LT2 lysate.

The transductions described so far were carried out with recipient strains lysogenic for wild-type phage P22. Ebel-Tsipis & Botstein (1971) and Susskind *et al.* (1971) reported that P22 codes for at least two distinct systems for exclusion of superinfecting DNA, controlled by the genes *sieA* and *sieB*. The gene *sieA* excludes, to some extent, DNA of transducing

Table 5. *Differential labelling of lysates of LD⁺ and LD strains*

Strain on which H5 was grown	No. of p.f.u. ml ⁻¹	³ H (c.p.m.)* ³² P (c.p.m.)
T4/2 (= LD ⁺)	1.3 × 10 ¹⁰	0.31
LD1.2	9.8 × 10 ⁹	0.36
LD2.2	1.9 × 10 ¹⁰	0.36
T4/2 (= LD ⁺)	8.8 × 10 ⁹	0.20
LD1.1	6.8 × 10 ⁹	0.12
LD2.1	5.6 × 10 ⁹	0.12

* ³H counts represent bacterial DNA in transducing particles; ³²P counts indicate phage DNA.

Table 6. *Efficiencies of plating of LD-grown H5 lysates and the ratios of abortive to recombinant transductants for hisB22⁺*

Strain on which H5 was grown	E.o.p. on indicator strain*		Abortive transductants
	LD	LT2	Recombinant transductants
LT2 (= LD ⁺)	1	1	13.0
LD10	1	0.27	15.4
LD1.1	1	0.21	12.6
LD1.2	1	0.19	4.5
LD2.1	1	0.80	33.0
LD2.2	1	1.60	8.7
LD3.1	1	2.70	7.2
LD3.2	1	2.10	29.0

* Titres of lysates on their LD host were set equal to 1; titres on LT2 are expressed as (p.f.u. on LT2)/(p.f.u. on LDx.y).

particles, in addition to homo- and heteroimmune phages. If DNA of LD donors were, for some unknown reason, more sensitive to the *sieA* system than DNA of the wild type, the LD effect could be explained by a stricter exclusion in the lysogenic recipient (hypothesis *c*). This hypothesis was tested using alysogenic recipients. P22c⁺ (wild type) lysates could have been used, but in order to avoid lysogenization of potential transductants by coinfecting phages, these transductions were carried out with clear-plaque phages. To prevent lysis of transduced alysogenic recipient cells by coinfecting lytic phages, transductions were performed at the very low m.o.i. of 0.05. Since the number of transductants was expected to be low, phage mutant HT13/4 was used. Superinfection of transduced bacteria by lytic phages and subsequent lysis on the selective plate was avoided by growing the transductants at 42 °C. P22 does not produce viable phages at this temperature since its head-tail assembly is temperature sensitive (Israel *et al.*, 1967). Observing these precautions, we compared the transduction frequencies for *hisB22⁺* of HT13/4:LT2 and HT13/4:LD10. Again the LD effect (reduction to 1.7%) was observed. Therefore we can exclude the hypothesis (*c*) that the P22 prophage in the recipient cells affects the transducing DNA of LD donors by superinfection exclusion or by some other protective mechanism.

Finally, the possible influence of an HMR system (Arber & Dussoix, 1962) was tested. The working hypothesis (*d*) implies that: (i) LD mutants are defective in a modification function (*m*⁻) and so also in the corresponding restriction (*r*⁻); if they were *m*⁻*r*⁺, their unmodified DNA would be degraded by their own restriction enzyme. (ii) This HMR system should affect transducing DNA but not the DNA of plaque-forming phages, since the e.o.p. values (Table 6) show no significant differences. (iii) In LD⁺ recipient strains, unmodified transducing DNA from LD donors should be degraded by their restriction enzyme.

If this hypothesis is correct, transduction efficiencies should not be decreased if recipient

Table 7. LD restriction effect on different *Salmonella* phages

Phage (grown on LD10)	No. of p.f.u. ml ⁻¹ on indicator strains			Restriction factor	
	LD10	4423	LT2	p.f.u. on LT2	p.f.u. on 4423
	($m_{LD}^- r_{LD}^-$)	($m_{SA}^- r_{SA}^-$)	($m_{LD, SA}^+ r_{LD, SA}^+$)	p.f.u. on LD10	p.f.u. on LD10
FO	4.0×10^9	5.4×10^9	3.4×10^9	0.85	1.35
9NA	1.6×10^8	1.8×10^8	1.0×10^8	0.63	1.12
A3	1.8×10^{10}	1.4×10^{10}	5.2×10^9	0.29	0.78
ES18h1	2.7×10^9	3.1×10^9	3.2×10^7	1.2×10^{-2}	1.15
L	9.0×10^{10}	1.0×10^{11}	1.1×10^9	1.2×10^{-2}	1.11
KB	2.1×10^8	2.7×10^8	6.7×10^5	3.2×10^{-3}	1.29
A1a	3.0×10^{10}	2.0×10^{10}	5.9×10^7	2.0×10^{-3}	0.67
Px1c2	6.4×10^9	not tested	1.0×10^5	1.6×10^{-5}	—

* These titres were assayed on strain 4420 ($m_{SA}^- r_{SA}^-$), since 4423 was resistant or immune to phages FO and ES18h1.

strains are also LD. As donor, we used a spontaneous *thy*⁺ revertant from LD1.1 and grew HT13/4 on it. As recipients, T4/2(P22) and LD1.1(P22) were used. LD donor and LD recipient were isogenic for the LD marker and LD and LD⁺ recipients were isogenic for *thy*. There was no reduction in the transduction frequency (T/P ratio) for *thy*-2⁺ from LD donor to LD recipient (1.5×10^{-5} compared with 8.0×10^{-6} for the LD⁺ donor to LD⁺ or LD recipients). The T/P ratio for the LD donor to LD⁺ recipient was, of course, reduced markedly (9.5×10^{-7}). Thus these findings are consistent with the prediction that the HMR system in LD strains is damaged.

Further confirmation of this hypothesis could be achieved by directly demonstrating the degradation of LD DNA by the restriction enzyme in LD⁺ cells. However, transducing DNA is affected to widely varying extents, depending on the gene product, and the expression of some genes is not restricted at all (Table 4). Therefore the overall rate of degradation may be low. This could explain why determinations of the lengths of re-extracted LD DNA from LD⁺ recipients did not yield meaningful information. Infective particles of P22, however, which would have homogeneous DNA molecules, are not restricted for some unknown reason. We therefore tested other *Salmonella* phages for their sensitivity to the LD system. Table 7 shows that e.o.p. values of LD-grown lysates on LD⁺ indicator vary between a restriction factor of 1 (i.e. no restriction) exhibited by the phages FO and 9NA and 1.6×10^{-5} (phage Px1c2).

Degradation of DNA in LD⁺ cells

We chose phage A1a, which is restricted to 4×10^{-4} , to grow ³H-labelled lysates on strains LD1.1 and T4/2 (= LD⁺). Strain T4/2, as a presumed restricting host, was infected with purified phages in the presence of 2 mM-KCN at an m.o.i. of 10. The KCN was removed by centrifuging, and the bacteria were resuspended in NB and incubated for 12 min at 37 °C. They were then lysed, and their DNA was extracted. Samples of these DNA preparations were sedimented, together with ¹⁴C-labelled P22 DNA as a reference, through 5 to 20% neutral sucrose gradients (Fig. 2*a, b*). In the same way, the non-restricting host LD1.1 was infected, and phage DNA was re-extracted and analysed (Fig. 2*c*). The DNA of LD-grown A1a phages became heterogeneous in size when injected into the LD⁺ strain. The peak fractions represent fragments of about 0.35 to 0.5 of the length of mature DNA. DNA of T4/2-grown phages, however, was not degraded. The results obtained for LD1.1-grown phages were similar to those for T4/2-grown phages when injected into LD1.1. These results establish the validity of our explanation of the LD phenomenon in terms of an HMR system.

Fig. 2. Sedimentation profiles through 5 to 20% sucrose gradients of ^3H -labelled DNA of phage A1a, grown in LD1.1 or T4/2 (= LD⁺), after injection and re-extraction from LD1.1 or T4/2. (a) A1a:LD1.1, extracted from T4/2; (b) A1a:T4/2, extracted from T4/2; (c) A1a:T4/2, extracted from LD1.1. ●, ^3H -labelled DNA of A1a; ○, ^{14}C -labelled DNA of P22 as reference.

Identity of the LD and SA systems

Three HMR systems are known in *Salmonella typhimurium*: LT (Zinder, 1960; Colson & Colson, 1967), SA (Colson & Colson, 1971) and SB (Colson & van Pel, 1974). Only the SA system is ineffective against phage P22. We therefore suspected that the LD and SA systems might be identical. Comparison of the e.o.p. values of the different LD-grown *Salmonella* phages plated on LD indicator and on an indicator strain $m_{\text{SA}}^-r_{\text{SA}}^-$ (strain 4423) favours this hypothesis (Table 7), since LD-grown phages which were strongly restricted by LT2 were not affected on the SA-defective indicator strain, and 4423-grown phages were not restricted in LD strains, whereas they were in LT2 (data not shown).

Further evidence for the common identity of the systems was obtained from locating the LD genes at the same position as SA genes. SA genes are 2 to 3% cotransducible with *pyrB* (Colson & van Pel, 1974). We therefore transduced strain SH480(P22), which is *pyrB*96, to prototrophy with HT13/4:LD10. Transductants were assayed for the LD phenotype by cross-streaking them against phage KB:LD10. LD isolates were lysed, but LD⁺ streaks grew because of the strong restriction effect on this phage. Some 2.2% of all *pyrB*96⁺ transductants were LD. The LD genes are therefore cotransduced with *pyrB* at the same frequency as the SA genes.

DISCUSSION

The initial purpose of this study was to complete our knowledge of the processes leading to the formation of transducing particles in lysates of *Salmonella* phage P22, with particular reference to the involvement of host functions. To this end, we isolated mutants (LD) with reduced donor ability. The LD phenomenon could be due to an alteration of a host-controlled process involved in the production of the transducing particles or it could be a consequence of events in the recipient cells.

To distinguish between these alternatives, the number of transducing particles was determined under circumstances eliminating the influence of adsorption, integration and expression in recipient cells. Differential labelling of DNA of transducing particles and of p.f.u. permitted this. These experiments excluded the possibility that fewer transducing particles are produced in LD strains after lytic infection with P22 than in wild-type hosts. However, this does not necessarily mean that bacterial functions are not involved in this process. The LD mutants studied represent a selected minority of all mutants isolated. They were chosen for practical reasons such as high yields of phages and strong reduction of T/P ratios. The fact that in LD-grown P22 lysates only transducing particles show reductions in the biological assay, whereas p.f.u. are little affected, indicated the need to investigate this phenomenon further. We excluded the possibilities that transducing DNA was lost by failure of integration into the recipient chromosome, or by destruction by one of the two known prophage-controlled systems for exclusion of superinfecting DNA (*sieA* and *sieB*), or by a hitherto unknown phage-controlled restriction mechanism.

All data presented can be interpreted in terms of a host-controlled modification-restriction (HMR) system (Arber & Dussoix, 1962; Dussoix & Arber, 1962). Such HMR mechanisms, which exhibit a protection function against foreign DNA, are widespread among bacteria (Boyer, 1971). Thus, it is suggested that LD strains are mutants in which the modification function is defective (m^-). Such mutants are only viable if the corresponding restriction function also is deficient (r^-). The DNA of LD mutants ($m_{LD}^- r_{LD}^-$) will be recognized in the LD⁺ recipient ($m_{LD}^+ r_{LD}^+$) as foreign DNA. The restriction enzyme will cleave this DNA and therefore reduce its biological activity. In the case of bacterial DNA injected by transducing particles, this will cause an increased probability of cleavage of the fragment. The number of transductants will be reduced significantly, although the actual number of transducing particles in the LD-grown lysates does not differ from that of a wild-type lysate. Since LD strains are (necessarily) also defective in restriction, they should not decrease the transduction frequency of LD-grown lysates when used as recipients in transduction. We have shown this to be so.

The hypothesis which explains the LD phenomenon in terms of a HMR system is also consistent with the results of other experiments:

1. Because of the random distribution of recognition sites for the restriction enzyme, it was anticipated that there must exist transducible DNA segments which do not carry any LD recognition site. This we showed for the fragments carrying the markers *metE* and *cysE* which were not affected by the LD restriction. Other markers were affected to different extents. This can be explained by different physical distances between the marker and the cleavage site of the restriction enzyme. If the distance is small, the probability of the marker being integrated into the recipient chromosome should be decreased drastically. With increasing distance of the cleavage site from the marker, the influence on integration should decrease. The unpublished observation that cotransduction frequencies for two markers located on the same LD-sensitive DNA fragment are usually decreased is consistent with this explanation.

2. The most direct demonstration that the LD phenomenon is due to degradation of unmodified DNA was obtained by measurement of its molecular weight after restriction. Because of its high sensitivity to LD restriction, unmodified DNA of phage A1a was

extracted from a restricting host 12 min after infection. At that time most of the infecting molecules had been cut into pieces that were 0.35 to 0.5 of the original length. LD⁺-grown, and therefore modified, DNA retained its original length in the same host.

3. We showed that p.f.u. of *Salmonella* phages other than P22 were sensitive to LD restriction when grown on a LD host. The different degrees of sensitivity may be explained by different numbers of recognition sites. It was surprising that the DNA of P22 p.f.u., which has the same length as transducing DNA (Schmieger, 1970) and as the DNA of sensitive phages such as A1a and L (data not shown), was not affected by the LD mechanism. Therefore the very strong restriction of phage Px1c2, a hybrid between P22 and another *Salmonella* phage, Py (Prell, 1970*a,b*), is remarkable, since approximately one-third of its genome is derived from P22 (Prell, 1975). Even more surprising is the restriction of phage L to 10⁻², since this phage is apparently isogenic with P22 except for the two immunity regions (Kahmann & Prell, 1971).

Colson & Colson (1971) described similar observations in their SA system. In favour of a presumed common identity of both systems was the absence of restriction of LD-grown *Salmonella* phages KB and A1a on an indicator deficient in SA restriction and vice versa. Further support for the common identity was obtained by showing that cotransduction of LD with *pyrB* occurred at the same frequency as Colson & Colson had found for SA.

We thank Miss U. Hamker for skilful technical assistance, the Deutsche Forschungsgemeinschaft for financial support, and C. Colson, P. E. Hartman, P. H. Mäkelä, H. H. Prell, K. E. Sanderson and B. A. D. Stocker for providing us with bacterial and phage strains.

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