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Transduction of Plasmid Determinants in *Staphylococcus aureus* and *Escherichia coli*

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Buoyant density analysis of transducing lysates derived from *Staphylococcus aureus* and *Escherichia coli* indicated that phage particles bearing plasmid determinants contain a quantity of DNA equivalent to that found in the lytic particles. Transducing particles that bear plasmid determinants smaller than viral DNA must therefore contain a quantity of DNA in excess of a single plasmid genome. In the *E. coli* P1*vir* system, a dependence upon host-mediated recombination for the transduction of small plasmids, but not for large R factors or chromosomal genes, was observed. However, no evidence for the involvement of such functions in the transduction of *S. aureus* plasmids was obtained. Although the origin of the additional DNA in plasmid transducing particles has not been identified, circumstantial evidence has been presented in the staphylococcal system indicating that transducing particles carrying a small tetracycline plasmid are not formed by the wrapping of multiple copies of this plasmid DNA.

According to the "wrapping choice" model of generalized transduction proposed by Ozeki and Ikeda (23), the ability of a phage to form transducing particles is attributed to the capacity of the phage coat to wrap bacterial DNA instead of its own phage DNA, a process with some resemblances to phenotypic mixing. Buoyant density analysis of transducing lysates of phage P1 (12, 32), phage P22 (9, 28), and staphylococcal phages (2, 7) has demonstrated that transducing particles carrying chromosomal determinants possess densities similar, if not identical, to the densities of the plaque-forming units (PFU), indicating that transducing and infectious particles contain the same quantities of DNA. This conclusion has been supported by direct physical measurements of the transducing DNA of phage P1 (12) and phage P22 (9, 26). These data suggest that, in generalized transduction, particles bearing bacterial genes contain a "headful" of DNA, that is, a fragment of DNA equal in length to the size of the viral genome.

The transfer of plasmids by generalized transducing phages appears to be similar in many respects to the transduction of chromosomal genes. However, investigations concerning the mechanisms of transducing particle formation have dealt exclusively with the transduction of chromosomal determinants, and it is not clear whether phage particles carrying plasmids are formed in a similar manner. Many plasmids that are efficiently transduced are significantly smaller in size than transducing viral genomes and do not by themselves constitute a headful of DNA. It therefore remains to be determined whether plasmids are indeed transduced via the wrapping choice mechanism, and, if so, how a headful quantity of plasmid DNA is generated for encapsulation by the transducing phage capsid.

The present work was undertaken to gain some insight into the nature of transducing particles carrying plasmid determinants, and involves a study of the generalized transduction of a penicillinase and a tetracycline plasmid in *Staphylococcus aureus* and the transduction of R plasmids in *Escherichia coli* by a virulent mutant of coliphage P1.

MATERIALS AND METHODS

Bacteria and bacteriophages. The staphylococcal strains used and their relevant characteristics are listed in Table 1. The penicillinase plasmid, pP81, is under stringent replication control, with one to two copies per chromosome. The pT81 plasmid is under relaxed replication control with 20 to 30 copies per chromosome (unpublished data). Group B staphylococcal phage 52HJD (hereafter called phage 52) of the International Typing Series was used in transduction studies because it is lytic for strain PS 81 (3). The molecular weight of staphylococcal group B phage DNA ranges from 29×10^6 to 31×10^6 (24). The *E. coli* K-12 strains used are listed in Table 2. All transductions of *E. coli* strains were performed with phage P1*vir*, a variant of P1*kc* that is unable to lysogenize *E. coli* K-12 strains. The molecular weight of this phage DNA has been calculated as being 73×10^6 (13). Phage stocks were prepared by the soft agar overlay method (4, 31).

Media. Stock cultures of staphylococcal strains

TABLE 1. *S. aureus strain characteristics*

Strain designation	Resident plasmids[a]			Strain derivation and other characteristics
	Designation	Relevant genotype[b]	Mol wt[c]	
PS 81	pP81	*asa*$^+$ *cad*$^+$ *mer*$^+$ *penI* *penZ*	18–21 × 10^6	Propagating strain for typing phage 81
	pT81	*tet*$^+$	2.7 × 10^6	
MU5	pP81	*asa*$^+$ *cad*$^+$ *mer*$^+$ *penI* *penZ*		Novobiocin resistant (*nov*$^+$) mutant of PS 81 selected after culture on media containing novobiocin
	pT81	*tet*$^+$		
PS 52				Propagating strain for typing phage 52; carries no known plasmid DNA
152 *rec-3*[d]				Recombination-deficient mutant of strain 152 isolated after treatment with nitrosoguanidine
MU6	pP81	*asa*$^+$ *cad*$^+$ *mer*$^+$ *penI* *penZ*		Strain 152 *rec-3* carrying pP81 plasmid as a result of transduction from PS 81
MU7	pT81	*tet*$^+$		Strain 152 *rec-3* carrying pT81 plasmid as a result of transduction from PS 81

[a] Plasmids have been previously described by Rubin and Rosenblum (25).
[b] Symbols indicate resistance to arsenate (*asa*$^+$), cadmium (*cad*$^+$), mercury (*mer*$^+$), tetracycline (*tet*$^+$), and the penicillinase structural gene (*penZ*) and regulatory loci (*penI*).
[c] Unpublished data.
[d] Obtained from R. V. Goering.

TABLE 2. *E. coli strain characteristics*

Strain designation	Plasmid			Reference and other characteristics
	Designation	Relevant genotype[a]	Mol wt	
RC180[b]	R28K	*amp*$^+$	44 × 10^6	Kontomichalou et al. (17)
RC181[b]	R6K	*amp*$^+$ *sm*$^+$	24 × 10^6[c]	Kontomichalou et al. (17)
RC172[b]	222/R3W	*cml*$^+$ *sm*$^+$ *su*$^+$	69 × 10^6	Nisioka et al. (20)
RC96[b]				Recombination-deficient (*recA*) mutant; carries no known plasmid DNA
MU1[d]	R6K	*amp*$^+$ *sm*$^+$		Strain RC96 carrying R6K plasmid
MU2[d]	R28K	*amp*$^+$		Strain RC96 carrying R28K plasmid
MU3[d]	222/R3W	*cml*$^+$ *sm*$^+$ *su*$^+$		Strain RC96 carrying 222/R3W plasmid
RC703[b]				K-12 strain with no known plasmid DNA
MU4				Maltose-negative mutant of RC703 selected after treatment with EMS[e]

[a] Symbols indicate resistance to ampicillin (*amp*$^+$), chloramphenicol (*cml*$^+$), streptomycin (*sm*$^+$), and sulfonamides (*su*$^+$).
[b] Strains obtained from R. C. Clowes.
[c] R. C. Clowes, personal communication.
[d] Strains derived by transduction from appropriate donors.
[e] EMS, Ethyl methane sulfonate.

were maintained on tryptone soy agar (Oxoid). Trypticase soy broth (BBL) was used as the standard nutrient medium for growth of cultures, to harvest and maintain phage stocks, and as a diluent in phage and bacterial assays. L agar and L broth (18) were used as standard nutrient media in *E. coli* experiments.

Transduction procedures. Transduction procedures were those described by Rubin and Rosenblum (25). In the staphylococcal system, cadmium-resistant transductants were selected on brain heart infusion agar (Difco) containing 2.5 ml/liter of 0.1 M $Cd(NO_3)_2 \cdot 4H_2O$, added after autoclaving. Antibiotic-resistant transductants were selected on brain heart infusion agar containing 5 μg of tetracycline hydrochloride per ml (Tetracyn, Roerig) or 5 μg of sodium novobiocin per ml (Albamycin, Upjohn). In the *E. coli* system, antibiotic-resistant transductants were selected on L agar containing 10 μg of sodium ampicillin per ml (Penbritin-S, Ayerst) or 100 μg of chloramphenicol per ml (Mann Research Laboratories). For selection of *mal*$^+$ transductants, eosin-methylene blue agar base (Difco) with 1% maltose was overlaid

with 0.8% saline to which 0.2 ml of the transduction mixture was added.

Density analysis of phage lysates. Lysates of staphylococcal phage 52 propagated on strain PS 81 were centrifuged in CsCl following the methods of Dowell and Rosenblum (7). The methods for density analysis of P1*vir* phage particles were those described by Ikeda and Tomizawa (12). Fractions (5 drops) of each CsCl gradient were collected from a hole punched in the bottom of the tube directly into 1.0 ml of sterile broth, and each fraction was assayed for PFU titer or transducing activity for plasmid or chromosomal determinants. In each experiment, a duplicate gradient was collected, and the refractive index of every third fraction was determined with an Abbé refractometer (Bausch and Lomb).

Isolation of plasmid DNA. R factor DNA was labeled, isolated, and examined by sedimentation through neutral 5 to 20% sucrose gradients following the techniques of Guerry et al. (11).

RESULTS

Buoyant density analysis of PFU and transducing particles. The buoyant densities of P1*vir* lytic and transducing particles were determined by centrifugation of phage particles in CsCl. The density distribution of such particles from a lysate prepared on strain RC180 is shown in Fig. 1. The infectious particles formed a peak at a density of 1.473 g/cm^3. The transducing particles carrying the *mal*$^+$ and *amp*$^+$ markers exhibited buoyant densities of 1.478 and 1.476 g/cm^3, respectively. The greater buoyant densities of the transducing particles in comparison to the lytic particles are consistent with differences in base composition between bacterial and viral DNA (12).

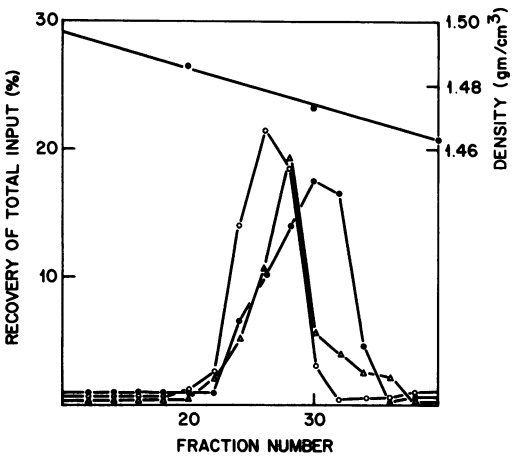

FIG. 1. *CsCl buoyant density gradient of P1vir transducing and lytic particles. Phage P1vir particles prepared on strain RC180 were pelleted by centrifugation, suspended in 3 ml of a CsCl solution in 0.01 M tris(hydroxymethyl)aminomethane (pH 7.5), and centrifuged for 20 h in a 50.1 rotor at 23,000 rpm and 15°C. Sixty 5-drop fractions of the resulting gradient were each collected into 1.0 ml of L broth. Each fraction was assayed for titers of lytic particles (●) and mal*$^+$ *(○) and amp*$^+$ *(△) transducing activity. Results, depicting fractions 10 to 40, are expressed as the percentage of activity recovered in each fraction compared with the total lytic or transducing activity added to the gradient.*

A similar analysis of a staphylococcal phage 52 lysate prepared on strain PS 81 is depicted in Fig. 2. A band of infectious particles, peaking sharply at a density of 1.528 g/cm^3, is seen. A definite peak of transduction of cadmium resistance (pP81) is observed. These transducing particles have an identical buoyant density distribution to the infectious particles, i.e., 1.515 to 1.528 g/cm^3, with the major activity coincident with the major peak of lytic particles. A similar distribution of transducing particles carrying the pT81 plasmid (data not shown) was observed.

The similarity of densities between the transducing and lytic particles indicates that both types of phage particles carry essentially equivalent amounts of DNA in each system.

Transduction of R factors from recombination-proficient and recombination-deficient *E. coli* strains. The involvement of host

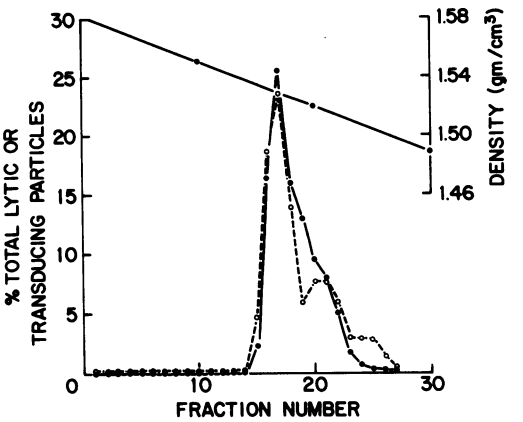

FIG. 2. *CsCl buoyant density gradient of staphylococcal phage and transducing particles. Phage 52 grown on PS 81 was suspended in 0.015 M tris(hydroxymethyl)aminomethane (pH 8.0), mixed with 3 ml of a CsCl solution dissolved in 0.015 M tris(hydroxymethyl)aminomethane (pH 8.0) to a refractive index of 1.38, and was centrifuged in a 50.1 rotor at 35,000 rpm and 15°C for 24 h. Sixty 5-drop fractions of the resulting gradient were each collected into 1.0 ml of trypticase soy broth from a hole punched in the bottom of the tube. Each fraction was assayed for PFU (●) and for cadmium (○) transducing activity using PS 52 as the indicator and recipient strain. The results, depicting fractions 1 to 30, are plotted as the percentage of activity in each fraction compared with the sum of activities from all fractions assayed.*

recombination functions in the transduction of chromosomal and plasmid genes in *E. coli* was studied. Of the three R factors investigated, two (R6K and R28K) are significantly smaller in size than the P1*vir* genome, and by themselves do not constitute a headful of DNA. The third plasmid, 222/R3W, is more nearly equivalent in size to P1 DNA.

The transduction rates of the plasmid markers and of a chromosomal marker from wild-type and *recA* donors are presented in Table 3. Assay of selected transductants indicated that the other plasmid-borne markers were cotransduced with the marker used for selection. The rates are the average of at least three separate experiments. These data suggest a requirement for host recombination function for the transduction of small R factor determinants, but not for the transfer of the chromosomal marker or the larger 222/R3W plasmid. The chloramphenicol resistance determinant was transferred at low frequencies from both the RC172 host and the *recA* mutant, but the rates in both cases were nearly equivalent. The chromosomal determinant was transduced from the *recA* mutant strain at significant levels, less than 10-fold lower than the frequencies observed from the RC180 and RC181 donors. In contrast, the transduction rates for the two smaller plasmids from the recombination-deficient donors were 1,000-fold lower than the rates seen from recombination-proficient host strains.

TABLE 3. *Transduction of plasmid and chromosomal determinants from recombination-proficient and recombination-deficient donors*[a]

Donor	Transduction rate[b]		
	mal$^+$	*amp*$^+$	*cml*$^+$
RC172 *rec*$^+$			2.1×10^{-8}
MU3 *rec*$^-$			1.1×10^{-8} (0.52)
RC181 *rec*$^+$	7.7×10^{-6}	1.6×10^{-5}	
MU1 *rec*$^-$	8.2×10^{-7} (0.11)	1.5×10^{-8} (0.001)	
RC180 *rec*$^+$	5.4×10^{-6}	2.1×10^{-6}	
MU2 *rec*$^-$	7.7×10^{-7} (0.14)	4.0×10^{-9} (0.002)	

[a] Phage P1*vir* was the transducing phage, and strain MU4 was the recipient in all transductions. The multiplicity of infection in all experiments was 0.2.

[b] Results are based on the number of transductants per PFU of phage P1*vir* titered on the donor strain and are averages of three separate experiments. Transductants were selected as follows: *mal*$^+$ on eosin-methylene blue agar with 1% maltose; *amp*$^+$ on L agar with 100 μg of sodium ampicillin per ml; *cml*$^+$ on L agar with 100 μg of chloramphenicol per ml. Numbers in parentheses indicate the ratio of transductants from *rec*$^-$ donors to those from *rec*$^+$ donors.

Isolation of *E. coli* R factor DNA. It was of interest to compare the sedimentation characteristics of R factor DNA derived from recombination-proficient and recombination-deficient strains. Plasmid DNA was isolated from cells labeled with [^{3}H]thymidine and was sedimented on 5 to 20% neutral sucrose gradients. The sucrose gradient profile of the R28K plasmid isolated from strain RC180 is shown in Fig. 3A. Two peaks of DNA were observed, inferred to be the covalently closed circular form (the more rapidly sedimenting peak) and the open circular form of this plasmid. The sucrose gradient profile of R28K DNA derived from strain MU2, a transductant clone that received this plasmid from strain RC180, is presented in Fig. 3B. Two peaks of DNA were apparent that banded in the same region of the gradient as those observed from the recombination-proficient donor

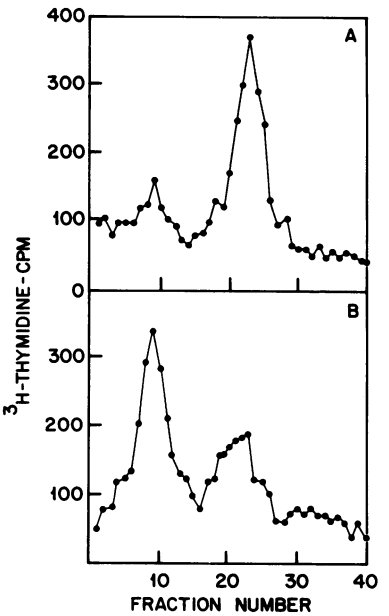

FIG. 3. *Neutral sucrose gradients of R28K DNA isolated from strains RC180 (A) and MU2 (B). Cultures of strains RC180 and MU2 were incubated with [^{3}H]thymidine, and the cells were lysed with sodium lauryl sulfate and mixed with NaCl to a final concentration of 1.0 M. After storage overnight at 4°C, the lysates were centrifuged at 17,000 × g and 0°C for 30 min. Samples (0.1 ml) of the supernatant fluids were mixed with equal volumes of 0.1 M tris-(hydroxymethyl)aminomethane–0.001 M ethylenediaminetetraacetic acid (pH 8.1) to lower the NaCl concentration and layered on 5 to 20% sucrose gradients containing 0.5 ml NaCl–0.01 M potassium phosphate (pH 7.0). The gradients were centrifuged in a 50.1 rotor at 45,000 rpm and 5°C for 108 min, were collected from a hole punched in the bottom of the tube, and were prepared for scintillation counting.*

strain. Examination of R6K DNA isolated from strain RC181 and a *recA* transductant for R6K (gradients not shown) revealed a similar pattern: two peaks of plasmid DNA in each gradient with similar sedimentation properties. The only notable variation between the sucrose profiles from Fig. 3A and 3B is the dominance of the inferred closed circular DNA species in the *rec*$^-$ strain and the inferred open circular DNA species in the *rec*$^+$ strain. This observation was also made for the R6K plasmid in the wild-type and mutant hosts. The reason for this difference is not clear.

These data indicate that the size of the R factor DNA does not differ significantly when isolated from *rec*$^+$ parent and *rec*$^-$ constructed strains. Thus, whereas the formation of transducing particles carrying small plasmids is a recombination-dependent event, the dissociation of plasmid DNA from transducing vector DNA in the *rec*$^-$ clones occurs in the absence of host-mediated recombination functions.

General characteristics of the staphylococcal transduction system. The rates of transduction of resistance to novobiocin, a chromosomal marker, and cadmium and tetracycline, plasmid-associated markers, were studied. The results of several transductions mediated by independently derived lysates of phage 52 propagated on strain MU5 are presented in Table 4.

All three genetic determinants were transferred at rates characteristic of generalized transduction. Resistance to novobiocin was transduced from MU5 at relatively low rates, in the range of 10^{-7} to 10^{-8}. A comparable level of transfer was observed in an homologous cross using the novobiocin-sensitive PS 81 recipient. Transduction of the plasmid markers occurred at rates higher than those observed for chromosomal gene transfer. The average transduction rate was in the range of 10^{-6} for the *cad*$^+$ marker and in the range of 10^{-5} for the *tet*$^+$ determinant. Transductants resistant to either cadmium or tetracycline served as competent donors of these markers in further transductions and at rates similar to those presented in Table 4.

TABLE 4. *Transduction of chromosomal and plasmid genes by staphylococcal phage 52*[a]

Lysate	Transduction rate[b]			MOI
	nov$^+$	*cad*$^+$	*tet*$^+$	
1		5.0×10^{-7}	1.2×10^{-6}	0.09
2	1.0×10^{-8}	1.0×10^{-5}		0.11
3	5.4×10^{-8}		2.6×10^{-6}	0.26
4	4.9×10^{-7}			0.10
5	2.8×10^{-7}			1.00
		1.4×10^{-5}	5.7×10^{-5}	0.20
6		7.3×10^{-6}	1.4×10^{-5}	0.24
7		7.3×10^{-6}	2.0×10^{-5}	0.10
8		2.6×10^{-6}	4.0×10^{-6}	1.50
		5.1×10^{-6}	1.0×10^{-5}	0.15
9		1.0×10^{-6}	1.5×10^{-5}	0.20

[a] The donor strain was MU5, and the recipient strain was PS 52. MOI, Multiplicity of infection.

[b] Results are expressed as the number of transductants per PFU of phage 52 titered on MU5. Transductants were selected as follows: *nov*$^+$ on BHIA with 5 μg of sodium novobiocin per ml; *cad*$^+$ on brain heart infusion agar with 2.5×10^{-4} M $Cd(NO_3)_2 \cdot 4H_2O$; *tet*$^+$ on brain heart infusion agar with 5 μg of tetracycline hydrochloride per ml.

Transductants selected for either *cad*$^+$ or *tet*$^+$ were screened for the presence of other plasmid-associated markers. These data are summarized in Table 5. Of 2,400 cadmium-resistant colonies tested, none carried tetracycline resistance. Of the 100 cadmium-resistant transductants tested, all were resistant to penicillin, arsenate, and mercury, at levels characteristic of the resistance levels of the donor PS 81 strain. If the initial selection was for penicillin resistance, cotransduction of the *cad*$^+$, *asa*$^+$, and *mer*$^+$ markers was observed in all clones screened. These data indicate that the pP81 plasmid is transferred intact by phage 52. However, no cotransfer of the pP81 and pT81 plasmid genes was observed.

In this system, like other generalized transducing systems, a dissociation between lysogeny and the transfer of bacterial genes was observed. PS 52 transductants selected for resistance to novobiocin, tetracycline, or cadmium were invariably nonlysogenic and were sensitive to infection with phage 52.

Plasmid transduction from recombination-deficient *S. aureus* donors. The rates of transduction of the tetracycline and penicillinase plasmids from recombination-proficient and deficient donor strains were determined, and these data are summarized in Table 6. The pP81 plas-

TABLE 5. *Cotransduction of penicillinase and tetracycline plasmids*

Transductants screened (selected markers)		Number resistant (unselected markers)[a]				
cad$^+$	*tet*$^+$	*cad*$^+$	*asa*$^+$	*mer*$^+$	*pen*$^+$	*tet*$^+$
2,400						0
100			100	100	100	0
	3,380	0				
	200	0	0	0	0	

[a] Sensitivity to cadmium (*cad*$^+$), arsenate (*asa*$^+$), and mercury (*mer*$^+$) determined by disk method of Novick and Roth (22). Sensitivity to penicillin (*pen*$^+$) and tetracycline (tet$^+$) tested with 10-U and 30-μg disks (BBL), respectively.

TABLE 6. *Transduction of cadmium and tetracycline resistance from recombination-proficient and recombination-deficient strains*[a]

Donor	Transduction rate[b]		MOI
	cad$^+$	*tet*$^+$	
PS 81 *rec*$^+$	5.3×10^{-6}	1.1×10^{-5}	0.16
MU6 *rec*$^-$	2.8×10^{-7}[c]		0.25
	2.9×10^{-7}		0.48
	2.0×10^{-7}		0.56
MU7 *rec*$^-$		3.5×10^{-5}[c]	0.12
		1.9×10^{-5}	0.33

[a] PS 52 and phage 52 were the recipient and transducing phage in all experiments. MOI, Multiplicity of infection.

[b] Results are based on the number of transductants per PFU of phage 52 titered on the donor strain.

[c] Results are from independently derived lysates of phage 52 propagated on MU6 and MU7.

mid was transduced from the *rec*$^-$ strain at a rate of 2.6×10^{-7}, in comparison to a level of 10^{-6} from the wild-type strain (PS 81). This rate, although lower than the average rate from the PS 81 donor, is near the transduction frequency occasionally seen in PS 81 × PS 52 crosses (Table 4) and is well within the range of rates characteristic of generalized transduction. The small pT81 plasmid was transduced from the *rec*$^-$ donor at rates entirely comparable to those seen from the PS 81 donor (10^{-5} per phage particle). Transductants receiving the plasmids from the mutant donors were identical in all respects to those obtained from wild-type donors.

Effects of UV irradiation upon staphylococcal transduction kinetics. In this study, the inactivation by UV irradiation of transducing activity for the tetracycline and penicillinase plasmids was compared. Samples of transducing lysates of phage 52 propagated on strains PS 81, MU6, and MU7 were irradiated for varying time periods, and the lytic and transducing activities of each sample were determined.

The effects of UV irradiation upon the transduction of the *cad*$^+$ and *tet*$^+$ markers from strain PS 81 are depicted in Fig. 4. As expected, both the PFU titer and the transduction rates for the plasmid-associated markers decreased with increasing doses of UV. This inactivation of transducing activity is typical of plasmid determinants (1). The transduction rate for the tetracycline plasmid decreased in an exponential fashion. In contrast, the transducing frequency for *cad*$^+$ did appear to be somewhat more resistant to inactivation; no effect upon transduction rates for the pP81 plasmid for the first 4 min of exposure was observed, after which an exponential decrease occurred.

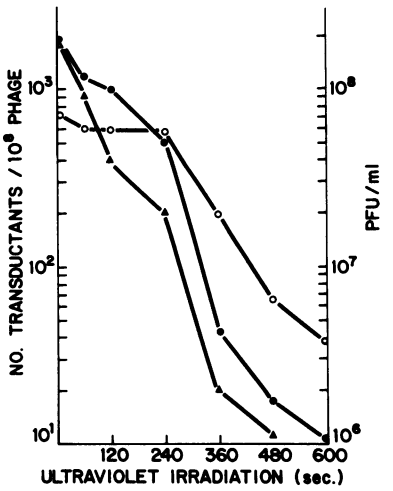

FIG. 4. *Effect of UV irradiation on the transduction of cadmium and tetracycline resistance. The genetic cross was PS 81 × PS 52. Samples (5 ml) of trypticase soy broth lysates of phage 52 were placed in 10-cm petri dishes and exposed to UV light for varying time intervals at a dose rate of 0.25 J/m² per s. Each sample was titered for PFU (△) and for transducing activity for cadmium (○) and tetracycline (●) resistance.*

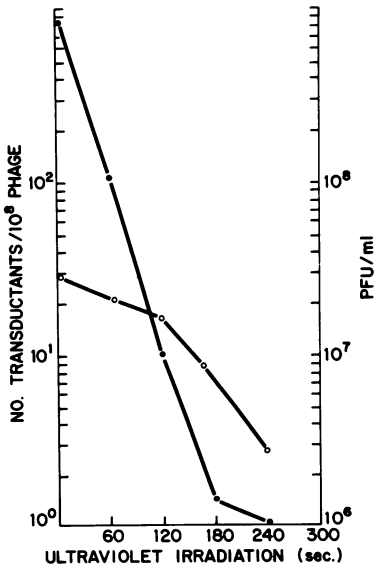

FIG. 5. *Effect of UV irradiation on the transduction of cadmium resistance. The genetic cross was MU6 × PS 52. Procedures were those described in Fig. 4, except that the UV dose rate was 0.5 J/m² per s. (●) PFU; (○) cadmium-resistant transductants.*

Similar data for phage 52 grown on the recombination-deficient strains carrying the penicillinase plasmid (Fig. 5) and the tetracycline plasmid

(Fig. 6) are presented. The dose rate in these experiments was 0.5 J/m^2 per s, double that used in the preceding study. The transducing activity for cadmium resistance exhibited a response to irradiation similar to that seen in Fig. 4, i.e., a very slow decrease in transduction rate for the first minute of irradiation, followed by a sharper exponential decline. The transducing particles carrying the tetracycline plasmid derived from the mutant host appeared to be somewhat more resistant to UV than those derived from strain PS 81. The rate of inactivation is comparable in this case to that seen for the pP81 plasmid.

DISCUSSION

This study has confirmed the observation that staphylococcal plasmids and R factors are transduced efficiently and with many characteristics similar to the generalized transduction of chromosomal genes. We have demonstrated from buoyant density analysis of the transducing lysates that plasmid-transducing particles contain a quantity of DNA similar to that found in lytic phage particles. Transducing particles for plasmids smaller than the genome of the transducing phage must therefore contain a quantity of DNA in excess of the single plasmid genome.

The production of transducing particles for the *E. coli* plasmids R6K and R28K seems to depend upon host-mediated recombination functions to produce a headful of DNA, since transduction of these plasmids from *recA* donors is about 1,000-fold lower than from recombination-proficient donors. Transduction of chromosomal determinants from *recA* donors is only moderately reduced. The dissociation of the plasmid from the extra DNA in the recipient cells seems to be independent of the host *rec* system, since *recA* and *rec*$^+$ recipients are transduced at equivalent rates (data not shown), and the plasmid DNA isolated from a *recA* transductant is similar in sedimentation profile to the plasmid present in the donor. The large 222/R3W plasmid is transduced from a *rec*$^+$ donor at a low rate, characteristic of transfer of other plasmids only from the *rec*$^-$ strains. However, this rate is not reduced when a *recA* donor is used. Because its size is close to that of the DNA of the transducing phage P1*vir*, one can speculate that it is encapsulated, albeit inefficiently, in a transducing particle without requiring additional DNA.

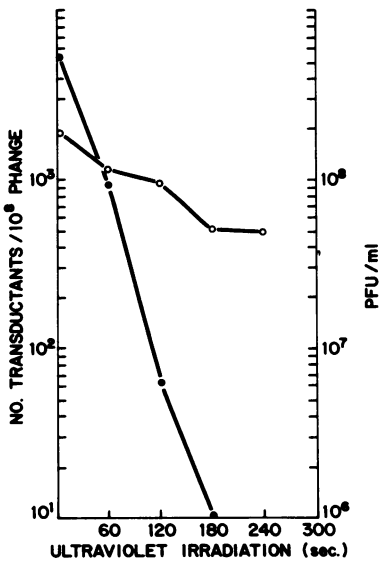

FIG. 6. *Effect of UV irradiation on the transduction of tetracycline resistance. The genetic cross was MU7 × PS 52. Procedures were those described in Fig. 4, except that the UV dose rate was 0.5 J/m^2 per s.* (●) *PFU;* (○) *tetracycline-resistant transductants.*

These data support the conclusion that recombination is necessary for the production of headful quantities of small *E. coli* plasmids, as would be predicted if plasmids were transduced via the wrapping choice mechanism of Ozeki and Ikeda (23). However, no evidence for host-directed recombination function in the transduction of staphylococcal plasmids could be obtained, although the *S. aureus rec*$^-$ donor used is functionally similar to *E. coli recA* mutants. These results were not unexpected, since other workers have reported the transduction of small tetracycline and chloramphenicol plasmids and larger penicillinase plasmids from a recombination-deficient *S. aureus* strain with properties similar to those of strain 152 *rec-3* (30, 34). However, evidence has been presented for both plasmid and phage-associated recombination systems in *S. aureus* (34), and therefore the involvement of recombination functions in staphylococcal plasmid transduction cannot be ruled out.

The origin of the additional DNA in plasmid-transducing particles has not been determined. The differential labeling of pre- and post-infection DNA during the preparation of a lysate would provide a means of identifying the origin of transducing DNA. The use of bromouracil (12), a heavy-density thymine analog, for this purpose was an approach that could not be used in either the staphylococcal or *E. coli* systems. Bromouracil or bromodeoxyuridine proved to be too toxic to *S. aureus* strains to permit labeling of bacterial DNA or the propagation of virus in its presence (unpublished data). In addition, the staphylococcal pT81 plasmid, as well as the

R6K and R28K factors, continue to replicate after infection of the host cells with each of the transducing phages (32a), making such a selective labeling of either host or viral DNA in these systems of little value. At the moment, therefore, the association of plasmid DNA during transduction with other plasmids, with chromosomal DNA, or with viral DNA must be considered. with chromosomal DNA, or with viral DNA must be considered.

The joint transfer of independent plasmids (10, 15) is the exception rather than the rule, and the occasional observations of such transductions may be a reflection of the close physical proximity of two plasmid types in donor cells. However, such reports do indicate that more than one plasmid molecule may be incorporated into a phage capsid, and therefore the packaging of multiple copies of such determinants to equal a headful of DNA is a distinct and appealing possibility. In this event, the transducing activity for the pT81 plasmid should not be inactivated by single-hit UV kinetics such as those described by Novick (21) for the inactivation of lytic particles and penicillinase transducing activity. Rather, this could lead to a multihit type of UV survival curve for transduction of this plasmid, and pT81 transducing particles should be more resistant to UV inactivation than those particles carrying the larger pP81 plasmid. The UV inactivation data, while somewhat ambiguous, does not seem to strongly favor multiple copy packaging.

Cotransduction of plasmid-associated markers with chromosomal determinants has not been reported in any well-studied transduction system. The low frequency of transfer in generalized transduction makes the detection of such joint transfers difficult, however, and at the moment this explanation for the formation of plasmid transducing DNA cannot be ruled out. Lastly, plasmid DNA may be transferred in association with viral DNA. If this is the case, the contribution of viral DNA must be extremely defective, since viral functions are not detectable in many transductant clones. This may be the most likely mechanism, since the identification of phage-associated recombination functions in some transducing phages (5, 29, 34) and the isolation of high-frequency transducing phage lines that involve stable associations between viral and plasmid genomes are well documented (6, 8, 14, 16, 20).

The formation of generalized transducing particles is a relatively rare event, and it appears that factors other than the size of the DNA fragments may influence the selection of DNA in the encapsulation process. Mutants of phages P22 (27) and P1 (33) have been isolated that exhibit increased abilities to mediate the generalized transduction of chromosomal determinants. The identification of phage-genetic functions governing the selection of DNA for encapsulation should add valuable insight into how bacterial markers, whether of chromosomal or plasmid origin, are occasionally incorporated into normal phage capsids.

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