Co-Transduction of Plasmids Mediating Resistance to Tetracycline and Chloramphenicol in *Staphylococcus aureus*

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Independent plasmids mediating resistance either to tetracycline (Tc) or chloramphenicol (Cm) were transduced successively into *Staphylococcus aureus* strain 8325. From this doubly resistant donor strain, Tc was co-transduced with a frequency of 40 to 50% when Cm was selected. Co-transduction of Cm was 5 to 10% with Tc selection. Plasmid elimination was infrequent and restricted to the Cm plasmid. A variant, doubly resistant strain gave 100% co-transduction of Tc and Cm and a high rate of joint elimination of both plasmid markers. Co-transduction of the plasmids from recombination-deficient donor strains was much reduced if the plasmids had been introduced separately into the donor strain, but occurred at the normal high rate if they had been introduced jointly. The plasmids were co-transformed at relatively low rates with closed circular deoxyribonucleic acid (DNA) from doubly resistant donors, but not with DNA from a mixed lysate of singly resistant strains. Our evidence favored a hypothesis of recombination-dependent, reversible linkage between the two plasmids as the basis for their co-transduction. Examination of plasmid DNA from the doubly resistant strains by ultracentrifugal and electron microscopic methods did not disclose any physical differences between singly and doubly resistant strains that might account for the observed co-transduction.

A number of drug resistance determinants are borne on plasmids in *Staphylococcus aureus* (18, 26). Genetic, ultracentrifugal, and electron microscopic evidence has established the occurrence of plasmids mediating resistance to tetracycline, chloramphenicol, and the production of penicillinase. Somewhat less complete evidence exists for plasmid determinants for resistance to streptomycin, erythromycin-lincocycin, and kanamycin-neomycin. With a few exceptions (18), each of these determinants is borne on a separate plasmid, as is shown by their independent transduction or elimination from multiply resistant cells. In a few instances two or more resistance determinants may be durably associated on a single plasmid, for example, penicillinase production and erythromycin resistance (18, 21). In other instances, some of these markers, notably those for resistance to tetracycline and streptomycin, have been co-transduced from strains of *S. aureus* natively resistant to these agents, but the co-transduced markers were not linked stably because they segregated independently in further transductions (11, 12, 14). Two possible explanations have been advanced for the latter results (12). One is that the plasmids undergo reversible association. The other is that one phage particle may include and co-transduce independent, unlinked plasmids that are in a state of close apposition.

We have attempted to gain more insight into this phenomenon by studying the co-transduction and co- transformation of tetracycline and chloramphenicol resistance plasmids from constructed, doubly resistant strains of *S. aureus*. Our genetic evidence suggests that the plasmids acquired linkage by a process involving recombination, but we have been unable to obtain biophysical evidence of the mechanism involved.

**MATERIALS AND METHODS**

**Bacterial strains and media.** The source and properties of the staphylococci and their plasmids are listed in Table 1. We used the designations Cm, Tc, Sm, and Km for the phenotypes of resistance to chloramphenicol, tetracycline, streptomycin, and kanamycin.

CY broth, 1%, a yeast extract-casein hydrolysate medium, was prepared according to Novick (24). Other media were commercial products.

**Plasmid elimination.** For elimination of plasmids, two to three colonies of a culture grown on a brain heart infusion (BHI; Difco) agar plate containing 25 µg
of chloramphenicol per ml, 10 μg of tetracycline per ml, or both antibiotics, according to the resistance pattern of the strain in question, were inoculated into 15 ml of 1% CY broth. The culture was grown at 43 C for 24 h with vigorous orbital shaking. Dilutions were plated on BHI agar, incubated overnight at 37 C, and replica-plated to BHI-antibiotic agar. The replica plates were read after 24 h at 37 C.

**Transduction.** The transductions were performed by published methods with minor modifications (5). For strains 8325 and its derivatives, the transducing agent was phage φ11, induced in the donor strain by ultraviolet irradiation. For strain 112 and 112UVS-1, typing phage 80 was used, grown by the soft-agar method (1). Recipient strains were mixed with phage at a multiplicity of 0.01 to 1.0. After incubation for 0.5 h at 37 C, the bacteria were sedimented by centrifugation, washed with 2.5 ml of BHI-0.02 M sodium citrate broth, suspended in 10 ml of the same broth, and incubated for 2.5 h at 37 C with orbital shaking. During this time the number of staphylococcal colony-forming units increased two- to fourfold. The cultures were plated, in 0.1-ml portions, on BHI-citrate agar containing 10 μg of tetracycline, 25 μg of chloramphenicol, or 100 μg of kanamycin per ml. The posttransductional incubation caused slight decrease in the absolute number of Cm or Km transductants but increased the number of Tc transductants about 1,000-fold.

**Transformation.** The transforming deoxyribonucleic acid (DNA) was the isopropanol-extracted DNA isolated by isopnic centrifugation (details given below). DNA was assayed spectrophotometrically at 260 nm. The recipient cells were strain 8325 or 8325 bearing a transduced Sm chromosomal marker. The transformation procedure followed those described for plasmid transformation in *Escherichia coli* and *S. aureus* (6, 20). A culture was grown overnight on BHI agar at 37 C, washed off, and suspended in 1% CY to an optical density equivalent to 1.25 x 10^8 colony-forming units/ml. The cell suspension was then diluted 10-fold in 1% CY broth and incubated with orbital shaking at 37 C. When the optical density of the culture reached a level equivalent to 2.5 x 10^9 colony-forming units/ml, it was centrifuged at 4 C. The cells were washed and suspended in ice-cold 0.1 M CaCl_2. Plasmid DNA was added to give a final concentration of 10^8 colony-forming units of cells and 1.0 μg of DNA per ml. The mixture (1 ml) was incubated for 60 min in an ice bath. It was then transferred to a 42-C water bath for 2 min with intermittent shaking, chilled again in ice water, and then diluted 10-fold in 1% CY broth. The diluted mixture was incubated for 2.5 h at 37 C without shaking. The cells were collected by centrifugation, resuspended in 1% CY broth, and spread on selective BHI agar plates identical to those used for transduction. Other dilutions were plated on BHI agar to determine the viable counts. The plates were incubated for 48 h at 37 C. In later experiments tryptic soy broth (Difco) replaced the CY broth. The controls in all transformation experiments included (i) cells carried through the transformation procedure without DNA, and (ii) treatment of DNA with deoxyribonuclease I (50 μg/ml made up in 5 mM MgCl_2) (Worthington Biochemical Corp.) for 20 min prior to the addition of competent cells. Antibiotic-resistant progeny did not appear in any of the control experiments. Furthermore, when we used a chromosomally Sm recipient, the progeny were all Sm, whereas the DNA was always prepared from streptomycin-susceptible donors.

**Table 1. Description of staphylococci and plasmids**

<table>
<thead>
<tr>
<th>Designation</th>
<th>Relevant properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>8325</td>
<td>Propagating strain for typing phage 47. Harbors three prophages, φ11, φ12, φ13 (25).</td>
</tr>
<tr>
<td>RN450</td>
<td>Strain 8325 cured of its three prophages. Received from R. P. Novick (25).</td>
</tr>
<tr>
<td>RN1030</td>
<td>Mutant of RN450 deficient in recombination. Received from R. P. Novick (33).</td>
</tr>
<tr>
<td>pSH1</td>
<td>A plasmid mediating tetracycline resistance, transduced from <em>S. aureus</em> strain 55C1 (32).</td>
</tr>
<tr>
<td>pC22.1</td>
<td>A plasmid mediating chloramphenical resistance by means of chloramphenical acetyltransferase. Received in strain 8325 from W. V. Shaw (31).</td>
</tr>
<tr>
<td>pSH2</td>
<td>A transducible staphylococcal plasmid mediating kanamycin resistance. (P. W. Stifler, M. Schneider, and H. M. Sweeney, unpublished data)</td>
</tr>
<tr>
<td>112</td>
<td>A naturally occurring strain of <em>S. aureus</em>. Received from R. V. Goering (10).</td>
</tr>
<tr>
<td>112UVS-1</td>
<td>Recombination-deficient mutant of strain 112. Received from R. V. Goering (10).</td>
</tr>
<tr>
<td>SH570</td>
<td>Strain 8325 bearing plasmids pSH1 and pC22.1 as the result of separate transductions.</td>
</tr>
<tr>
<td>SH720</td>
<td>Strain 8325 bearing plasmids pSH1 and pC22.1 as the result of co-transduction from SH570.</td>
</tr>
<tr>
<td>SH720a</td>
<td>Spontaneous variant of SH720.</td>
</tr>
</tbody>
</table>
Density gradients. The S. aureus strains were labeled with either methyl-3H-thymidine (50 Ci/mmol) or 2-14C-thymidine (56 mCi/mmol) (New England Nuclear) for at least two generations in 1% CY broth. The labeled cultures were lysed by a modification of the procedure of Novick and Bouanchaud (27; R. P. Novick, personal communication). The washed cells were suspended in 2.5 M NaCl + 0.05 M disodium ethylenediaminetetraacetic acid (EDTA), pH 7.0, to 2 × 10^9 cells/ml and treated with lysostaphin (Schwarz/Mann) at 50 μg/ml for 15 min at 37 C with gentle shaking. The resulting protoplasts were then lysed with 1.5 vol of a lytic mixture containing 1% Brj 58, 1% sodium deoxycholate, and 0.05 M disodium EDTA, pH 7.5. After standing for 10 min at room temperature, this crude lysate was centrifuged at 16,000 rpm for 45 min at 4 C. The supernatant (cleared lysate) was either frozen at -70 C until ready for examination or used immediately for preparative buoyant density equilibrium centrifugation. For this purpose the cleared lysate was mixed with CsCl to give a final density of 1.54 g/ml and ethidium bromide (EtBr) was added to a final concentration of 300 μg/ml. The mixture was centrifuged in a polyallomer tube at 43,000 rpm for 24 h at 20 C in a type 65 rotor in a Spinco L5-65 centrifuge. The tube was punctured, and 18-drop (~0.2-ml) fractions were collected. Samples of μleters from each fraction were spotted on filter-paper squares that were then dried and washed sequentially with 250 ml each of cold 5% trichloroacetic acid, 95% ethanol, and anhydrous ether. The dried samples were counted in a scintillation spectrometer. The fractions containing the plasmid material were pooled, extracted twice with 1.5 ml of isopropanol, and then dialyzed against buffer containing 0.019 M NaCl, 0.0015 M sodium citrate, and 0.01 M disodium EDTA, pH 7.0. Samples of the dialyzed plasmid DNA were analyzed on 5.0 ml, 20 to 31% linear sucrose density gradients containing either 1 M NaCl and 0.05 M disodium EDTA, pH 7.5, or 1 M NaCl, 0.01 M disodium EDTA, and 0.3 M NaOH, pH 12.5. The DNA was allowed to be denatured 30 min on top of the alkaline gradients prior to centrifugation. The gradient was centrifuged in an SW39 rotor at 37,000 rpm for 180 min at 20 C. The tube was punctured, and 10-drop samples were collected directly on filter-paper squares that were dried and prepared for counting for radioactivity as described above.

Electron microscopy. To convert circular, covalently closed (CCC) DNA molecules to their open circular forms, the dialyzed plasmid DNA was dialyzed against 0.15 M ammonium acetate, pH 6.0. It then received 9,000 rads of X-ray irradiation. The irradiated plasmid DNA was then prepared for examination in the electron microscope by the micro-drop technique of Lang and Mitani (19). Copper-wire grids (200-mesh) coated with collodion were used to support the DNA-protein monolayer. The DNA-containing grids were rotary shadowed with platinum-palladium (80-20%). The grids were examined in an RCA electron microscope. The contour lengths of the open circular DNA molecules were measured with a map measurer from photographic enlargements. Similar enlargements of a replica of a Fullam grating (54,864 lines/inch) were used as a measurement standard.

RESULTS

Transduction of Tc and Cm. Strain SH570 was obtained by the successive transduction of the Tc plasmid pSH1 and the Cm plasmid pC22.1 into strain 8325 (Table 1). From strain SH570 the two resistance plasmids were co-transduced with a frequency of 44% when Cm was selected and 6.8% when Tc was selected (Table 2). One co-transductant clone, selected for Cm, was designated strain SH720 and was used in further experiments as our standard donor strain. Although the frequency of transduction was highly variable, the proportions of singly to doubly resistant progeny obtained by transduction from strain 720 or from other co-transductant strains were similar to those from the original strain 570. Thus, the co-transduced markers were not durably associated. The frequency of co-transduction was not altered by variation in the multiplicity of phase infection in the range of 0.01 to 1.0. Similar results were obtained with different Cm and Tc determinants introduced into 8325 or other host strains and with the use of other transducing phages. A Km plasmid, pSH2 (molecular weight, ~9 × 10^6, copies per cell ~2) was transduced into strain 720 (P. W. Stiffler, M. J. Schneider, H. M. Sweeney, and S. Cohen, unpublished data). From this host, pSH2 could be transduced to 8325 at a frequency of 0.4 × 10^-4 without any co-transduction of Tc or Cm (<0.05%). The latter two markers showed their usual frequency of co-transduction with Cm or Tc selection, but Km was never co-transduced under these circumstances (<0.014% with Cm selection and <0.001% with Tc selection). Since the φ11 genome has a molecular weight of ~28 × 10^6 it should have been able to accommodate both pSH2 and several copies of pSH1 and pC22.1 (molecular weight, ~3 × 10^9) (27, 30). The lack of co-transduction of pSH2 suggests that the co-transduction of pSH1 and pC22.1 is not caused simply by random packing of available DNA in phage heads.

One strain of 720, detected by chance and designated SH720a, had distinctive properties. From this strain, Tc was co-transduced with a frequency of 100% when Cm was selected and Cm was co-transduced with a frequency of 10.8% when Tc was selected (Table 2). These frequencies did not change in serial transductions from co-transductant progeny.

Strain 720a also differed from 720 with respect to frequency of elimination of its resist-
Table 2. Co-transduction of plasmid-mediated resistance to tetracycline and chloramphenicol

<table>
<thead>
<tr>
<th>Donor strain</th>
<th>Frequency of Cm transduction*</th>
<th>Co-transduction of Tc% (%)</th>
<th>Frequency of Tc transduction*</th>
<th>Co-transduction of Cm% (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>570</td>
<td>$3.0 \times 10^{-9}(0.66 \times 10^{-9})$</td>
<td>44 (8.7)</td>
<td>$5.0 \times 10^{-9}(2.4 \times 10^{-9})$</td>
<td>6.8 (1.7)</td>
</tr>
<tr>
<td>720</td>
<td>$1.4 \times 10^{-9}(1.1 \times 10^{-9})$</td>
<td>41 (4.5)</td>
<td>$2.6 \times 10^{-9}(1.8 \times 10^{-9})$</td>
<td>4.7 (1.4)</td>
</tr>
<tr>
<td>720a</td>
<td>$0.21 \times 10^{-9}(0.124 \times 10^{-9})$</td>
<td>100 (0)</td>
<td>$8.2 \times 10^{-9}(10 \times 10^{-9})$</td>
<td>10.8 (2.5)</td>
</tr>
</tbody>
</table>

* Results are means of multiple experiments and are expressed as number of transductants per plaque-forming unit of φ11, induced by ultraviolet irradiation and titered on strain RN450. The multiplicities of infection ranged from 0.01 to 1.0. Values in parentheses are standard deviations.

Table 3. Elimination of Tc and Cm plasmids from strains 720 and 720a

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of colonies tested</th>
<th>Tc Cm (%)</th>
<th>Tc Cm* (%)</th>
<th>Tc* Cm* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>720</td>
<td>4,568</td>
<td>99.5b</td>
<td>0.5*</td>
<td>0</td>
</tr>
<tr>
<td>720a</td>
<td>1,246</td>
<td>18</td>
<td>50</td>
<td>32</td>
</tr>
</tbody>
</table>

b. Signifies susceptible.

* Rates of elimination of Tc or Cm from 8325 bearing either plasmid singly resembled those for strain 720.

The high frequency of co-transduction of pSH1 and pC22.1 in 720, and especially in 720a, suggested that these originally independent determinants had acquired linkage. However, the segregation pattern in serial transductions suggested that the linkage, if present, could be broken easily. Furthermore, the strikingly decreased stability of Cm in 720a and its relatively high rate of loss with Tc suggested that one or both plasmids in this strain had undergone a mutation that affected their stability or that of the putative recombinant plasmid.

Acquisition of linkage between the Cm and Tc markers presumes some type of recombination that might depend on host cell, plasmid, or phage functions. We therefore repeated the foregoing experiments in two recombination-deficient (Rec-) strains of S. aureus. Strain 112UVS-1 was isolated by Goering and Pattee (10) as an ultraviolet-sensitive mutant that gave no transductants for a chromosomal marker (novobiocin resistance) but retained the ability to give transductants for tetracycline or oleandomycin resistance, presumably plasmid markers. Strain RN1030 was an ultraviolet-sensitive mutant of a his auxotroph of strain RN450 (33). It gave plasmid transductants at a somewhat reduced rate, but could not be transduced for his and was very deficient in its production of recombinants between pairs of compatible or incompatible penicillinase plasmids (33). In addition, we found that neither of these ultraviolet-sensitive strains served as transduc-
markers in S. aureus afforded another method of testing for the establishment of linkage between pSH1 and pC22.1, namely, by co-transformation. Using strain 8325 as the recipient, we obtained 660 transformants/ml with 1.0 µg of pSH1 DNA per ml and 590 transformants/ml with 1.0 µg of pC22.1 per ml. The number of transformants was two to ten times greater with recipient cells grown in tryptic soy (Difco) broth than in CY broth. The number of pSH1 transformants increased approximately linearly with increasing concentration of plasmid DNA from strain 720 to a maximum at 1.0 µg/ml data not shown). In confirmation of Lindberg and Novick (20), we found that 8325 was a competent recipient for transformation of pSH1 but its prophage-cured derivative RN450 was not. RN450 was made an effective recipient by lysogenizing it with φ11 phage. Similarly, we were able to show that the cells transformed with pSH1 or pC22.1 plasmids had acquired a CCC DNA duplex plasmid that was indistinguishable from that of the donor strain with respect to equilibrium centrifugation in CsCl-EtBr, velocity sedimentation in neutral sucrose gradients, and contour length in electron micrographs (see below).

Co-transformation of two distinct markers provides acceptable evidence of their linkage if the possibility of their dual transformation by independent DNA molecules can be excluded. This is accomplished best by comparing the number of single and double transformants obtained with varying concentrations of DNA. If the slope of the curve for the number of double transformants parallels that for the single transformants, the two markers may be considered to be linked on the same DNA molecule (13). In view of the low rates of transformation in our system, such a demonstration seemed to be impracticable. Instead, we compared the number of co-transformants obtained with the same amount of DNA from a mixed lysate of equal portions of strain 8325(pSH1) and 8325(pC22.1). Table 5 indicates that pSH1 was transformed from each of the three plasmid DNA pools at roughly comparable rates, whereas pC22.1 was transformed several times more readily from 720 plasmid DNA than from the other two sources. We have no explanation for this difference.

Both Tc and Cm were co-transformed with 720 plasmid DNA, albeit at frequencies much lower than those of their co-transduction. With 720a plasmid DNA, the frequency of co-transformation of Cm with Tc was very low, only 0.21%, but the frequency of co-transformation of Tc was 100% among 11 Cm transformants. When the resistance determinants were transduced from the co-transformed strains, the frequencies of co-transduction of Tc and Cm were comparable to those of 720 or 720a, according to the source of the DNA with which the donor strain had been transformed. Joint elimination of the co-transformed plasmids and their co-transduced progeny was similarly related to the source of the transforming DNA. In contrast, co-transformation was not observed with plasmid DNA from the mixed lysate of the singly resistant strains. If we may assume that the proportion of pSH1 to pC22.1 DNA in the mixed lysate was similar to that in strains
720 and 720a, then this experiment supports a hypothesis of linkage of the plasmids in 720 and 720a.

Density gradient centrifugation and electron microscopy of pSH1 and pC22.1 DNA. In an attempt to detect physical evidence of recombination between pSH1 and pC22.1 plasmids, we analyzed the CCC plasmid content of the singly resistant strains by pycnographic centrifugation in CsCl-EtBr density gradients. Strain 8325 contained no detectable CCC DNA (Fig. 1A), but when it harbored pSH1 or pC22.1, the dye-buoyant density gradient exhibited a dense peak characteristic of CCC DNA (Fig. 1A). The plasmid peak derived from 720 or 720a resembled that of pSH1 DNA (Fig. 1B). The less dense peak contained chromosomal fragments and any linear or open circular plasmid molecules that were present. The CCC plasmid DNA was about 0.75% of the total cell DNA for the pC22.1 strain and about 5% for cells bearing either the pSH1 plasmid or both plasmids.

The CCC-DNA molecules isolated from these strains were co-sedimented on neutral sucrose gradients with differentially labeled CCC-ColEl DNA which has a sedimentation coefficient of 23s (2) (Fig. 2). The calculated sedimentation coefficient for each plasmid was 20s, corresponding to a molecular weight of approximately $3 \times 10^4$ (Fig. 2A and 2B, respectively), in good agreement with earlier results of Novick and Bouanchaud (27). Recombination between the two plasmids might take the form of co-integration, analogous to integration of lambda phage into the E. coli chromosome (4). Such a recombinant plasmid would be expected to have a genome mass of $6 \times 10^4$ daltons or a sedimentation coefficient of about 27s (3). In Fig. 2C and 2D one can see that the plasmid DNA of strain 720 or 720a had a peak of radioactivity at 20s, the same as the individual resistance plasmids (Fig. 2A and B). The leading edge of the plasmid peaks extended through fraction 23, where a 27s plasmid peak should have been found. There was no evidence of a separate fraction peaking in this region. If the recombinant plasmids were unstable when isolated by pycnographic methods, the survival of a small number of recombinants might have been masked in these gradients. To test for this possibility, we examined the plasmids in the electron microscope. Equal amounts of the pSH1, pC22.1, and the 720 and 720a plasmid DNA were centrifuged separately through 20 to 31% neutral sucrose gradients. Three pools were made of fractions 20 to 23, 24 to 26, and 27 to 29, designated as the leading, peak, and trailing pools, respectively. The pools were dialyzed against 0.15 M ammonium acetate, pH 6.0. X-ray irradiated, shadowed with platinum, and examined in the electron microscope. Each plasmid pool contained small, open circular DNA molecules (Fig. 3). Their properties are listed in Table 6. The average contour lengths of pSH1 DNA and that from the 720 and 720a plasmids appeared to be the same and slightly smaller than that of pC22.1 DNA (Table 6). The DNA pools of the leading and peak fractions of each preparation contained a few large, open circular DNA molecules, approximately twice as long as the principal DNA component. These large molecules, which we took to be plasmid concatemers, were too few in number to determine accurately their proportion to the smaller DNA component. They did not appear to be more numerous in the 720 or 720a DNA preparations. We concluded that, if recombinant plasmids were recovered in the centrifugation procedures, they were not sufficiently different in contour length from the bulk of the plasmid molecules to be detected by our procedures.

**DISCUSSION**

Investigation of the co-transduction of Sm and Tc from S. aureus E169 by Grubb, O'Reilly, and May (12) disclosed that Tc and Sm had properties of plasmid markers and that they were co-transduced at frequencies of 2 to 44%. Tc and Sm segregated independently in the co-transductant progeny and, therefore, did not

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**Table 5. Co-transformation of resistance to tetracycline and chloramphenicol with plasmid DNA**

<table>
<thead>
<tr>
<th>Source of plasmid DNA</th>
<th>Selected marker</th>
<th>Transformants/ ml</th>
<th>No. of transformants isolated</th>
<th>No. of co-transformants isolated</th>
<th>Co-transformation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>720</td>
<td>Tc</td>
<td>603</td>
<td>1,281</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cm</td>
<td>39</td>
<td>120</td>
<td>8</td>
<td>6.7</td>
</tr>
<tr>
<td>720a</td>
<td>Tc</td>
<td>394</td>
<td>946</td>
<td>2</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Cm</td>
<td>4</td>
<td>11</td>
<td>11</td>
<td>100</td>
</tr>
<tr>
<td>Mixture of 8325(pSH1)</td>
<td>Tc</td>
<td>470</td>
<td>863</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>and 8325(pC22.1)</td>
<td>Cm</td>
<td>7</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Fig. 1. Equilibrium centrifugation of [3H]thymidine-labeled DNA from plasmid-free and plasmid-containing strains of S. aureus 8325. Cleared lysates of staphylococci were centrifuged separately to equilibrium in CsCl-EtBr solutions. Counts were normalized to the same incorporation to make them comparable. The separately centrifuged gradients were superimposed in the plot. (A) ×, 8325; O, 8325(pC22.1); ■, 8325(pSH1). (B) O, SH720; ■, SH720a.

Fig. 2. Sedimentation analyses of CCC DNA from strains of S. aureus centrifuged through 20 to 31% neutral sucrose density gradients. [14C]thymidine-labeled 23S ColE1 DNA, serving as a marker, was mixed with each sample. (A) 8325(pC22.1); (B) 8325(pSH1); (C) SH720; (D) SH720a. The arrow indicates the peak fraction of the CCC ColE1 DNA.
FIG. 3. Electron micrographs of open circular plasmid DNA molecules from *S. aureus.* (A) 8325(pC22.1); (B) 8325(pSH2); (C) SH720; (D) SH720a. Magnification, ×44,480. Bar represents 0.25 μm.

### Table 6. Contour lengths and molecular weights of co-transducible plasmids

<table>
<thead>
<tr>
<th>Source of plasmid DNA</th>
<th>$s_{20, w}$</th>
<th>No. of molecules measured</th>
<th>Mean length ± SE*(μm)</th>
<th>Estimated mol wt*c</th>
</tr>
</thead>
<tbody>
<tr>
<td>8325(pC22.1)</td>
<td>20</td>
<td>560</td>
<td>1.47 ± 0.01</td>
<td>3.04 × 10^6</td>
</tr>
<tr>
<td>8325(pSH1)</td>
<td>20</td>
<td>236</td>
<td>1.37 ± 0.02</td>
<td>2.84 × 10^6</td>
</tr>
<tr>
<td>720</td>
<td>20</td>
<td>155</td>
<td>1.37 ± 0.02</td>
<td>2.84 × 10^6</td>
</tr>
<tr>
<td>720a</td>
<td>20</td>
<td>67</td>
<td>1.36 ± 0.03</td>
<td>2.82 × 10^6</td>
</tr>
</tbody>
</table>

*a* $s_{20, w}$, Sedimentation coefficient corrected to water at 20 C.

*b* SE, Standard error.

*c* Molecular weights were calculated by assuming a mass of 2.07 × 10^6 daltons per μm (4).

appear to be determined by a single plasmid. Grubb and his colleagues (11) suggested that the Tc and Sm plasmids had either undergone a readily reversible association by recombination or that they might be situated in close apposition and therefore be co-transduced as separate plasmids by inclusion in a single phage particle. The hypothesis of reversible recombination was based on a similar proposal made by Richmond to explain some observations in *S. aureus* strain 147 containing two compatible, i.e., dissimilar, penicillinase plasmids (29). Al-
though the plasmids were usually stable in this staphylococcus, a few clones showed a high rate of elimination of both plasmids, a high rate of plasmid recombination, and a high (28 to 84%) frequency of plasmid co-transduction. Richmond suggested that the plasmids underwent recombination and that the combined plasmids were transduced as a single unit. In the light of current knowledge of the genomic size of staphylococcal penicillinase plasmids and transducing phage (30), it is doubtful that a single phage particle could include two penicillinase plasmids. Nevertheless, although a more complex hypothesis may be needed to explain the co-transduction of penicillinase plasmids in Richmond's experiments, the basic assumption of plasmid recombination may be valid and offers a precedent for a comparable interaction of other dissimilar plasmids. Furthermore, the staphylococcal Tc plasmid is so small that, even though the size of the Sm plasmid is unknown, it is likely that both plasmids could be accommodated in a single phage virion.

The co-transduction of a different set of independent plasmids, a Col plasmid and an R factor, has also been reported (8). The co-transduction of pSH1 and pC22.1 in our experiments resembled the earlier results with the Tc and Sm plasmids and seems likely to be of similar origin. In our case the genetic experiments suggest that co-transduction was based upon linkage due to some type of readily reversible recombination. It must be admitted that, in view of the small size of these plasmids, a hypothesis of joint inclusion of randomly chosen, independent plasmids by phage multiplying in strain 720 is also tenable. From our data and with the use of $5.5 \times 10^{-15}$ g as the normal DNA content of a staphylococcus (27), we find that strain 8325(pSH1) contained a minimum of 63 copies of pSH1 and strain 8325(pC22.1) contained, similarly, 8.3 copies of pC22.1. Assuming that the transducing phage could accommodate $28 \times 10^4$ daltons of DNA randomly chosen from plasmid DNA or pieces of chromosomal or phage DNA of similar size, we calculate, on the binomial theorem, that a phage containing one copy of pC22.1 DNA would have a probability of 0.35 of containing, in addition, at least one copy of pSH1 DNA. Likewise, a phage containing one copy of pSH1 would have a probability of 0.056 of containing one copy of pC22.1. On this basis, random packaging of plasmid DNA could explain the general level of co-transduction from strain 720. However, such a hypothesis would not explain the absence of co-transduction of pSH2, an element easily encompassed within a φ11 virion. Furthermore, it would be necessary to make additional assumptions to explain the results with strain 720a. Such an assumption might be that pC22.1 in this strain was defective in ability to survive and was complemented in that respect by pSH1. The available precedents argue against such an event. A replication-defective staphylococcal penicillinase plasmid was not complemented by another penicillinase plasmid of a different incompatibility type (26). In E. coli, R factors failed to complement an Flac factor thermosensitive for replication (23). Similarly, a normal Flac plasmid failed to complement most thermosensitive replication mutants of ColE1, but a few mutants were complemented (15). Since pSH1 and pC22.1 were obviously of different incompatibility groups, the available evidence would point away from a hypothesis of complementation as an explanation for the properties of 720a. Furthermore, such a hypothesis would not easily explain the high rate of joint elimination of the plasmids from 720a. However, if the Cm and Tc markers in 720a were recombinant, that is, situated on a single plasmid, they might be retained in the cell by the maintenance locus of the Tc plasmid even if that for the Cm plasmid was defective. Potential segregants resistant only to chloramphenicol would not be expected to survive. The ready loss of both plasmids from strain 720a might be caused by partial impairment of the Tc maintenance locus as a result of recombination at an unusual site. On this basis, the Tc and Cm plasmids would be recombinant both in 720 and 720a, but would differ either in their sites of recombination or as a consequence of additional mutation in 720a.

The experiments with the Rec− strains also support a hypothesis of linkage by recombination between the Cm and Tc plasmids. The much reduced frequency of co-transduction of these plasmids from the Rec− host strains is to be compared with the nearly complete failure of RN1030 to mediate recombination between compatible penicillinase plasmids and between mutants of the same plasmid (33). Similarly, recombination between compatible R factors and between mutants of the same R factor was abolished in a recA− strain of E. coli, and was much reduced in recB and recC strains (9). Compatible staphylococcal plasmids in RN1030 recombined during transduction from RN1030, presumably through the mediation of genes of the vegetatively multiplying transducing phage (33). The low level of co-transduction of Tc and Cm from the Rec− staphylococci may have been caused also by phage genes. If that was the case, they substituted much less effectively for host
Rec function than in the experiments with penicillinase plasmids (33).

The co-transformation of Cm and Tc by plasmid DNA from strains 720 and 720a, but not from the mixed Cm and Tc cells, is further evidence of linkage between the DNA of the two plasmids. Such linkage is usually taken to signify covalent union between the co-transformed DNA.

Although the genetic evidence points to recombination between the plasmids, the biological studies to date are disappointing in showing no basis for recombination. One possibility that we entertained was that the plasmids were co-integrated reversibly, in a fashion analogous to the insertion and excision of lambda phage in _E. coli_. Such a reaction has been suggested for the reversible union of R-factor plasmids (4). The double-length molecules that were seen in the electron micrographs were obvious candidates, but they did not appear more numerous in 720 or 720a than in cells bearing single plasmids. On this hypothesis, all the pC22.1 plasmids in 720a should have been doubles, since in this strain transduction of Tc was regularly accompanied by co-transduction of Tc. We think we would have detected a frequency of this magnitude. It is of course possible that only a few of the recombinant plasmids survived the CsCl-EtBr centrifugation; they might therefore have been more infrequent in the electron micrographs.

Other genetic interactions between Tc and Cm plasmids, such as insertions, deletions, or reciprocal crossovers, might have been involved in our experiments. If they were, their effects on the contour lengths of the plasmids were not detectable by the methods employed, which are estimated to have a precision of ±3% (19).

Catenated CCC plasmid DNA has been detected in _E. coli_ bearing a Col plasmid or R factor and also in _S. aureus_ bearing a penicillinase plasmid (7, 16, 17, 28). The catenated forms of the Col and the penicillinase plasmids were detected in DNA extracted from multiplying cells. In these circumstances, the catenanes were evidently replicative intermediate forms. If catenanes could be formed between the dissimilar Tc and Cm plasmids used in our experiments, they might furnish a basis for their apparent linkage. The DNA used for our electron micrographic studies was taken from stationary-phase cells and therefore might not have been optimal for demonstrating plasmid catenanes. We hope to reexamine plasmid DNA taken from actively growing cells for evidence on this point when it becomes possible to return to this problem. For the present, the physical basis for the co-transduction and co- transformation of Cm and Tc plasmids remains uncertain.

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**LITERATURE CITED**


