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Generation of Transducing Particles in *Staphylococcus aureus*†

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Transduction of plasmid pC194 and bacteriophage φ11de varied inversely with the multiplicity of infection. As the multiplicity of infection decreased from 10^{-1} to 10^{-5} PFU/CFU, the transduction frequency of pC194 increased 10^4-fold; the transduction frequency of φ11de increased 300-fold with a 100-fold decrease in multiplicity of infection. Physical and genetic analysis of the transduced DNA showed that pC194 resided in the phage particle as a random, circularly permuted linear concatemer. In DNA prepared from phage that cotransduced pC194 and φ11de, pC194 resided in the transducing phage primarily as a linear multimer of 15.8 kilobases, or about 5.4 pC194 monomers. The pC194 multimer was randomly inserted into the φ11 genome.

Small staphylococcal plasmids are not transduced by generalized transduction, as are chromosomal determinants (2, 27). This was first shown by Grubb et al. (6, 7), who demonstrated cotransduction of staphylococcal streptomycin resistance and tetracycline resistance plasmids. Later, Stiffler et al. (26) and Iordanescu (10) extended this observation to include a variety of other small plasmids. These investigations highlight a dilemma that arises because cotransduction of plasmid DNA in *Staphylococcus* spp. often occurs at frequencies higher than predicted for a random packaging model. Transduction of a single plasmid species occurs at frequencies of about 10^{-5} to 10^{-8} per PFU; frequencies vary depending upon the plasmid used and the transducing phage (2). Other unselected plasmids are often cotransduced at between 1 and 10% of the single transduction frequency (9), even though the predicted cotransduction rates for randomly packaged plasmids should be the product of the probabilities of the single transductional events. This high rate leads to the suggestion that cotransducing plasmids are not randomly assorted, but associate in some specific way. Iordanescu (10) suggested that some staphylococcal plasmids become transiently associated during the formation of the transducing particle. Furthermore, this transient association is limited to specific pairs that share short regions of homology (14). Cotransduced plasmids generally resolve into individual replicons in the recipient cell, but occasionally (ca. 1%) the plasmid phenotypes remain associated as stable plasmid cointegrates that arise from site- and orientation-specific recombination (14). Plasmid cointegrates are stable in subsequent transductions or transformations and do not under normal circumstances dissociate into the component replicons (9).

Examination of these transient transductional associations has been complicated by the assumption that these events occur at relatively low frequencies in a transducing phage population. However, we have utilized the finding that plasmid transduction occurs at higher rates at very low multiplicity of infection (MOI) (19, 20) to directly analyze the transducing DNA in the phage particles.

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MATERIALS AND METHODS

Bacterial strains and plasmids. The bacteria and plasmids used in this study are listed in Table 1. Procedures for cultivation of these organisms and selection procedures for antibiotic resistance phenotypes have been described (4, 21, 22, 24).

Bacteriophage. The serogroup B bacteriophages φ11 and 29 were used. The phage propagation and the transduction protocol used in our laboratory have been previously reported (23). Concentrated φ11 was purified by CsCl density gradient centrifugation (17). The purified phage was disrupted by suspending the bands in 2% sodium dodecyl sulfate and extracting twice for 15 min at 37°C with equal volumes of redistilled phenol equilibrated with 0.1 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). The resulting DNA solution was then extracted twice with chloroform-isoamyl alcohol (24:1) and ethanol precipitated (4).

Plasmid purification. Preparation of cleared lysates for the purification of pC194 and the use of dye-buoyant density gradient centrifugation to purify covalently closed circular plasmid has been published (23). The procedure of Novick et al. (16) was used to prepare cleared lysates containing φ11 or pI258.

Hybridization analysis. Agarose gel electrophoresis and the procedures for Southern blot transfer and hybridization have been described by us (4). In certain instances, the double-blot procedure of Smith and Summers (25) was used to make mirror image blots of the same gel. Nick-translated plasmid DNA used as a probe was prepared by the method of Rigby et al. (18) with [α^{32}P]TTP. Probe specific activities varied between 5×10^7 and 1×10^8 dpm/μg of DNA. Restriction endonuclease digestions were performed as suggested by the manufacturer (New England Biolabs).

RESULTS

Effect of MOI on transduction frequency. As shown in Fig. 1, the transduction frequency of pC194 by φ11 was inversely proportional to the input MOI. At higher MOI (0.1 to 1 PFU/CFU), the transduction frequency was 10^{-7} to 10^{-6}, which is in the expected range for transduction in *Staphylococcus aureus* (6, 7, 10, 23, 24, 26). However, as the MOI decreased from 1 to 10^{-5} PFU per recipient, the transduction frequency increased dramatically to almost 10^{-2}. The effect of MOI on transduction frequency did not depend upon the transducing bacteriophage used, because we observed the same relationship when a related serogroup B phage, 29, was used (data not shown).

TABLE 1. *S. aureus* strains

Strain	Plasmid	Relevant phenotype[a]	Source
8325-4	None	None	R. P. Novick
8325-4(pC194)	pC194	Cm^r	R. P. Novick
8325-4(ϕ11de)	ϕ11de	Em^r	R. P. Novick
8325-4(pC194)(ϕ11de)	pC194, ϕ11de	Cm^r Em^r	(4)

[a] Cm^r, Chloramphenicol resistance; Em^r, erythromycin resistance. The antibiotic resistances are known plasmid determinants.

The transduction of ϕ11de was also dependent upon the input MOI (Table 2), yet at each MOI ϕ11de transduction occurred at higher frequencies than for pC194. When we examined transduction frequencies of phage lysates from strains in which ϕ11de and pC194 coresided, both transduction and cotransduction were subject to enhancement at low MOI [Table 2, 8325-4(pC194)(ϕ11de)]. In this experiment, pC194 was transferred at a higher frequency in cotransduction than in transduction at roughly the same MOI [8325-4(pC194) and 8325-4(pC194)(ϕ11de)]. Other experiments (see below) indicated that ϕ11de can enhance pC194 transduction by as much as 100- to 1,000-fold. We have also collected comparable data documenting this effect of MOI on transduction frequency with the plasmids pSN1 (a tetracycline resistance plasmid) and pUB110 (a kanamycin resistance plasmid) alone and in the presence of ϕ11de (data not presented).

As yet we do not understand the mechanism of this phenomenon. We have ruled out the possibility of the presence of killer particles by comparing survival of infected cells after infection with specific numbers of PFU; we have eliminated the possibility of the presence of many defective bacteriophage particles by electron microscopic examination of lysates; we have used Sephadex chromatography and dialysis of bacteriophage preparations to rule out the presence of high- or low-molecular-weight inhibitors of transduction at high MOI; and we have dismissed transduction by newly formed phage at very low MOI, because the number of transducing particles generated would be too few to account for the frequencies observed.

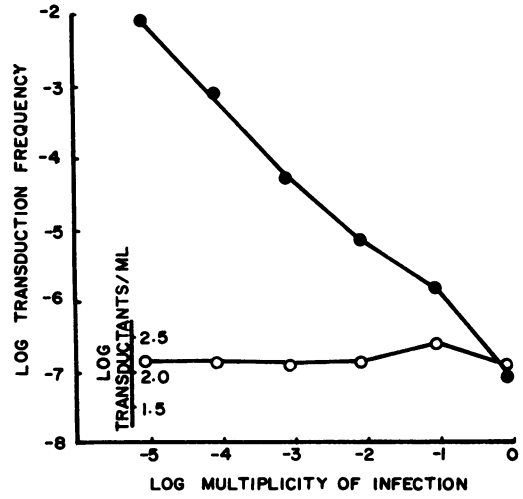

FIG. 1. Dependence of pC194 transduction on the MOI. A bacteriophage ϕ11 transducing lysate prepared on *S. aureus* 8325-4(pC194) was used to transduce strain 8325-4. The transduction frequency was calculated as the number of transductants per input PFU. Symbols: transduction frequency (●), transductants per milliliter (○).

Hybridization analysis of bacteriophage DNA. The high frequencies of transduction observed suggested that we should be able to use hybridization techniques to detect the presence of transducing DNA in the bacteriophage lysates. DNA was prepared from CsCl-purified bacteriophage preparations that transduced pC194 and ϕ11de, digested with restriction endonucleases, and blotted to nitrocellulose sheets. These blots were probed with nick-translated pC194 and, on occasion, with the nick-translated plasmid pI258 to identify the pI258-specific portion of ϕ11de (ϕ11de is a recombinant of pI258 and ϕ11 [13]).

When the pC194 probe was hybridized to undigested bacteriophage ϕ11 DNA, it bound strongly to the DNA of phage grown on strains 8325-4(pC194) and to 8325-4(pC194)(ϕ11de) and weakly to 8325-4(ϕ11de) (Fig. 2). The weak hybridization of pC194 to 8325-4(ϕ11de) is due to limited homology between pC194 and the pI258 region of ϕ11de (see below). The strong hybridization observed with the other two strains suggested that pC194 transducing DNA was either inserted into the phage genome or was present as a linear concatemer of the same size as mature phage DNA (43 kilobases [kb]). The latter possibility was eliminated when DNA from ϕ11 grown on 8325-4(pC194) was digested with *Eco*RI (an enzyme that does not cleave pC194) and then probed with pC194. The results (Fig. 2, lane 4) showed that the digested DNA had a considerable amount of homology to the plasmid, particularly in the 8- to 15-kb region of the gel and not at 43 kb. Figure 2 also shows that ϕ11 DNA from phage grown on a host that does not contain pC194 shares no appreciable homology with pC194 (compare lanes 5 and 7 with lane 4).

When DNA from bacteriophage ϕ11 grown on 8325-4(ϕ11de) was digested with *Eco*RI and hybridized with pC194, the probe identified a 12.8-kb band (Fig. 2, lane 5). Control experiments with pI258 as the probe showed that this band contained the pI258-specific region of ϕ11de and indicated that a significant degree of homology exists between pC194 and ϕ11de. However, when DNA from bacteriophage ϕ11 grown on strain 8325-4(pC194)(ϕ11de) was probed with pC194, a second band was seen at 15.8 kb (Fig. 2, lane 6). The size difference between the two bands is 3.0

TABLE 2. Effect of MOI on the transduction of staphylococcal plasmids pC194 and ϕ11de by bacteriophage ϕ11

Donor[a]	Plasmid	Selection[b]	MOI	Transduction frequency
8325-4(pC194)	pC194	Cm^r	8.4×10^{-1}	9.3×10^{-8}
	pC194	Cm^r	8.4×10^{-4}	7.4×10^{-5}
8325-4(ϕ11de)	ϕ11de	Em^r	6.1×10^{-1}	5.8×10^{-3}
	ϕ11de	Em^r	6.1×10^{-3}	1.4×10^{-2}
8325-4(pC194)(ϕ11de)	pC194	Cm^r	2.8×10^{-1}	1.4×10^{-7}
	ϕ11de	Em^r	2.8×10^{-1}	1.2×10^{-4}
	pC194, ϕ11de	Cm^r Em^r	2.8×10^{-1}	7.4×10^{-8}
	pC194	Cm^r	2.8×10^{-3}	1.6×10^{-4}
	ϕ11de	Em^r	2.8×10^{-3}	3.7×10^{-2}
	pC194, ϕ11de	Cm^r Em^r	2.8×10^{-3}	2.9×10^{-5}

[a] The recipient in all cases was *S. aureus* 8325-4, which provided an isogenic background for selection. The plasmids were established in the recipients as autonomously replicating entities.

[b] Cm^r, Chloramphenicol resistance; Em^r, erythromycin resistance. The antibiotic resistances are known plasmid determinants.

kb and would be predicted if pC194 (which is 2.91 kb [8]) had inserted into the 12.8-kb pI258 fragment of φ11de. Consequently, we wished to determine whether the 15.8-kb fragment was, indeed, the result of a pC194 insertion. DNA from bacteriophage φ11 grown on strains 8325-4(φ11de) and 8325-4(pC194)(φ11de) was digested with *Sau*96I, an enzyme that does not cleave pC194, but recognizes a number of sites on φ11de DNA, including several within the pI258 fragment. These digests were then probed with pC194. As shown in Fig. 2 (lane 7), *Sau*96I completely removed the 12.8-kb *Eco*RI fragment of φ11de while not affecting the migration of the 15.8-kb pC194-specific band (Fig. 2, lane 8). The 15.8-kb fragment is therefore a multimeric species of pC194 rather than the result of a single insertion of the plasmid into the 12.8-kb *Eco*RI fragment. These data indicate that pC194 integrates into the phage genome as random multimeric forms ranging from about 8 to 15 kb, but in the presence of φ11de the formation of the 15.8-kb multimer is preferred. However, this form is not exclusive because overexposure of such autoradiograms indicated a background smearing also in the 8- to 15-kb range.

UV inactivation. The presence of multimeric forms of plasmids in transducing particles suggests that the bacteriophage lysates should not display single-hit, first-order inactivation kinetics when subjected to UV irradiation. Indeed, pC194 and φ11de showed distinct differences in response to UV treatment. The transduction frequency of pC194 did not decline along with the decrease in PFU as the UV dosage was increased; rather transduction of pC194 actually increased about twofold (Fig. 3A). On the other hand, φ11de behaved characteristically as a plasmid packaged as a single species in the transducing particle. φ11de and the PFU declined in concert (Fig. 3B). In the φ11de-enhanced transducing lysate (Fig. 3C), pC194 transducing activity was also refractory to UV irradiation and remained relatively constant at increasing dose rates. Additionally, pC194 transducing frequencies were much higher (ca. 1,000-fold) than in the lysates prepared on a donor harboring pC194 alone. These results indicated (i) that φ11de displayed single-hit inactivation kinetics characteristic of monomolecular systems and (ii) that pC194 in both normal and enhanced transduction behaved as a multicomponent entity.

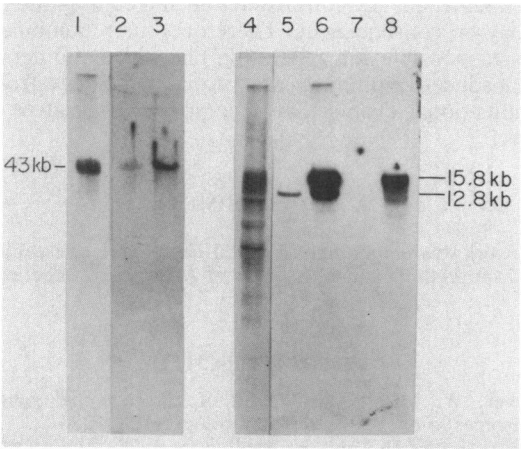

FIG. 2. Southern hybridization analysis of bacteriophage φ11 DNA probed with nick-translated pC194. Lanes: 1, undigested DNA of phage φ11 grown on strain 8325-4(pC194); 2, φ11 grown on strain 8325-4(φ11de); 3, φ11 grown on strain 8325-4(pC194)(φ11de); 4, 5, and 6, *Eco*RI-digested DNA used in lanes 1, 2, and 3, respectively; 7 and 8, *Sau*96I-digested φ11 DNA used in lanes 2 and 3, respectively.

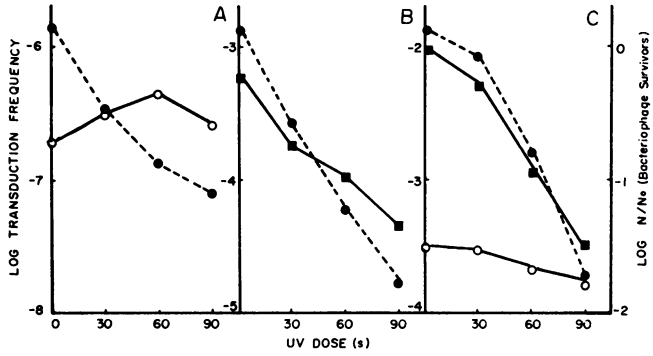

FIG. 3. Effect of ultraviolet irradiation of φ11 bacteriophage lysates on the transduction of pC194 and φ11de. (A) φ11 propagated on *S. aureus* 8325-4(pC194); (B) φ11 propagated on strain 8325-4(φ11de); (C) φ11 propagated on strain 8325-4(pC194)(φ11de). Each graph shows the response of PFU and transducing activity. Symbols: inactivation of φ11 PFU (●), transduction of pC194 (○) and φ11de (■).

When UV inactivation of bacteriophage lysates of φ11 that transduced pSN1 and pUB110 alone and in combination with φ11de were subjected to UV irradiation, a very similar response was seen, suggesting that these small staphylococcal plasmids are packaged similarly for transduction.

Hybridization analysis of plasmid multimers. The definitive proof of the presence of plasmid multimers in the bacteriophage DNA preparations was obtained from the following experiment. pC194 transducing DNA was digested with *Hpa*II (an enzyme which recognizes a single cleavage site in pC194). This treatment should generate monomer-sized molecules from multimers. When these digests were examined by hybridization with pC194, the DNA from bacteriophage grown on 8325-4(pC194) showed probe homology with a single 3-kb band (Fig. 4, lane 1). Contrast this finding to the same DNA preparation digested with *Eco*RI (Fig. 2, lane 4) in which the homology is between 8 and 15 kb. We did not observe any junction fragments that might correlate to a specific site of insertion even when these samples were run on 2% agarose gels. This suggests that the plasmid multimers are randomly inserted in the bacteriophage DNA.

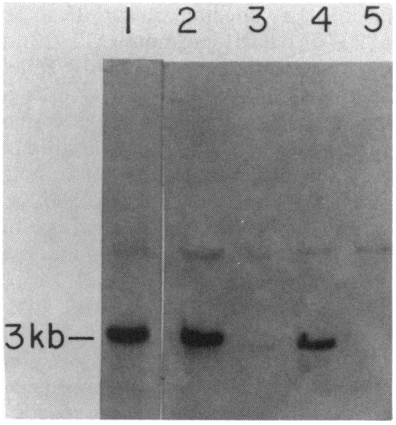

FIG. 4. Southern hybridization analysis of bacteriophage DNA probed with nick-translated pC194. Lanes: 1, 2, and 3 *Hpa*II-digested DNA of bacteriophage φ11 grown on *S. aureus* strains 8325-4(pC194), 8325-4(pC194)(φ11de) and 8325-4(φ11de), respectively; 4 and 5, *Hae*III digests of phage grown on 8325-4(pC194)(φ11de) and 8325-4(φ11de), respectively.

When *Hpa*II-cleaved DNA from bacteriophage φ11 grown on strains 8325-4(pC194)(φ11de) and 8325-4(φ11de) was analyzed, a 3-kb linear monomer was again seen. The faint 3-kb band identified by the pC194 probe in the digest of DNA from bacteriophage grown on 8325-4(φ11de) (Fig. 4, lane 3) was a portion of the pI258 region of the φ11de plasmid. This was shown by digesting the same two DNA samples with *Hae*III (an enzyme which also cleaves pC194 at a single site). The DNA sample from the bacteriophage grown on 8325-4(pC194)(φ11de) again showed the 3-kb monomer band (Fig. 4, lane 4), but no homology was seen (Fig. 4, lane 5) in the 3-kb region with the DNA from growth on 8325-4(φ11de).

DISCUSSION

The inverse relationship between transduction frequency and multiplicity of infection is not a new finding. Although contrary to expected relationships, others have observed exactly the same response in *S. aureus* (19) and in *Bacillus* spp. (20). In each case, however, the results have been dismissed as a curiosity. We have examined this in more detail, but the experiments represented in this communication do not provide an unequivocal explanation of this relationship.

After observing the same patterns with three different plasmids alone and in combination with φ11de and after considering the results of UV inactivation studies, it became apparent that the organization of DNA in the transducing particle was central to a final explanation. The high levels of transduction at low MOI indicated that many more transducing particles were present than previously assumed. Simple calculations based on these frequencies indicated that hybridiation analysis could be used to examine construction of the transducing particles generated in this system.

Hybridization studies of transducing particle DNA showed that pC194 is not packaged as an autonomously replicating plasmid, but is inserted into the bacteriophage DNA of the transducing particle in random oligomers. However, the presence of φ11de in the donor cell enhances the formation of pentamers of pC194. The bacteriophage portion of φ11de may provide the functions necessary for such discrete multimer formation.

Because we did not detect any bands that would represent pC194-φ11 junction fragments, we conclude that pC194 is not integrated into any specific site on these genomes. The genome of φ11 is circularly permuted (11, 12), and the lack of specific junction fragments probably reflects that the pC194 concatemers contained in the transducing particles are covalently attached at random to the phage genome. Therefore specific junction fragments should not be present. Coupled with the observation that pC194 homology migrated with the main band of bacteriophage DNA in undigested samples, we conclude that the formation of a plasmid transducing particle is the result of random covalent association of the plasmid multimer and φ11 DNA.

This hypothesis finds support from the UV inactivation data. pC194 transduction was surprisingly resistant to the effects of UV irradiation; in fact, the frequency actually increased after irradiation. The relative insensitivity of pC194 is understandable if each transducing particle contained more than one copy of pC194. This conclusion contrasts with that of Ubelaker and Rosenblum (27), who indicated that multiple copies of plasmids are not packaged in staphylococcal transducing particles. However, inspection of their UV inactivation data indicates that the decline in transducing activity did not closely follow the decline in plaque-forming bacteriophage and may indicate that the plasmids they studied were present as multiple copies in the transducing particles. In contrast, the UV inactivation kinetics of φ11de followed the decline in PFU very closely. Assuming that the maximum amount of DNA in a transducing particle is similar to the 43-kb φ11 genome (3, 12), φ11de (which is about 33 kb) cannot reside in more than one complete copy in the transducing particle. Consequently, the different response of pC194 and φ11de transduction to UV inactivation suggests that these two plasmids are transduced as different physical entities.

Transduction of chromosomal determinants is usually increased by UV irradiation (1). This is generally thought to be a consequence of enhanced recombination of the incoming UV damaged DNA by an induction of the SOS functions of the recipient (1). The stability of plasmid transduction may indicate that certain recipient recombination functions are involved in the generation of stable plasmid transductants. This possibility is important since other studies have concluded that plasmid transduction is unaffected by the recombination proficiency of the recipient (28). However, this should not be taken to mean that recombination systems are not involved in plasmid transduction. Wyman et al. (28) have shown that a bacteriophage recombination system may function during the transduction process. Moreover, pC194 can take part in *rec*-independent recombinational events (5), implying that it may have transposon-like properties. These considerations indicate that, after introduction of the plasmid concatemer, recombination functions may play a role in the establishment of pC194. This might also explain the process of plasmid cointegrate formation. Intermolecular recombination between different linear plasmid multimers after cotransduction could be responsible for the formation of cointegrate structures that have the appropriate site and orientation specificity (14, 15). Although this may provide an explanation for the manner in which cointegrates are formed, recombination in the recipient probably does not explain high-frequency plasmid cotransduction or the specificity that such cotransduction exhibits (i.e. pE194 and pT181 are cotransduced at high frequencies, but pE194 and pC194 are not, even from donors that contain all three plasmids [10]). This specificity implies a transient plasmid association of which high-frequency cotransduction and cointegrate formation may be consequences. Therefore, the recombinational events responsible for generating plasmid monomers from the transduced multimers are probably distinct from the recombinational events that accompany associative transduction.

ACKNOWLEDGMENTS

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