Role of Staphylococcal Phage and SaPI Integrase in Intra- and Interspecies SaPI Transfer

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Received 21 April 2007/Accepted 21 May 2007

SaPIbov2 is a member of the SaPI family of staphylococcal pathogenicity islands and is very closely related to SaPIbov1. Typically, certain temperate phages can induce excision and replication of one or more of these islands and can package them into special small phage-like particles commensurate with their genome sizes (referred to as the excision-replication-packaging (ERP) cycle). We have studied the phage-SaPI interaction in some depth using SaPIbov2, with special reference to the role of its integrase. We demonstrate here that SaPIbov2 can be induced to replicate by different staphylococcal phages. After replication, SaPIbov2 is efficiently encapsidated and transferred to recipient organisms, including different non-Staphylococcus aureus staphylococci, where it integrates at a SaPI-specific attachment site, attS, by means of a self-coded integrase (Int). Phages that cannot induce the SaPIbov2 ERP cycle can transfer the island by recA-dependent classical generalized transduction and can also transfer it by a novel mechanism that requires the expression of SaPIbov2 int in the recipient but not in the donor. It is suggested that this mechanism involves the encapsidation of standard transducing fragments containing the intact island followed by int-mediated excision, circularization, and integration in the recipient.

Pathogenicity islands (PTIs), a subset of horizontally transferred genetic elements now known generically as genomic islands, are largely responsible for the difference in overall pathogenic potential between otherwise closely related enteric organisms. In gram-positive bacteria, the role of PTIs seems to be somewhat different; pathogenesis is largely due to constant chromosomal genes, and PTIs are more frequently involved in diseases caused by single protein toxins. With this perspective, we considered a remarkable family of highly mobile PTIs in Staphylococcus aureus that are induced to excise and replicate by certain resident prophages, are encapsidated into small-headed phage-like particles, and are transferred at frequencies commensurate with the plaque-forming titer of the phage (17). Late in infection, a SaPI-specific DNA band appears in an agarose gel containing whole-cell lysates and represents monomeric SaPI DNA that is released from the small-headed particles (11). This process is referred to as the SaPI excision-replication-packaging (ERP) cycle, and the high-frequency SaPI transfer is referred to as SaPI-specific transfer (SPST) to distinguish it from classical generalized transduction (CGT) (see below).

The SaPIs have a highly conserved genetic organization which parallels that of bacteriophages and clearly distinguishes them from all other horizontally acquired genomic islands (16). The SaPI1-encoded (17) and SaPIbov2-encoded (25) integrases are required for both excision and integration of the corresponding elements, and it is assumed that the same is true for the other SaPIs. The known SaPIs are mostly 14 to 17 kb long, and many carry superantigen or other virulence genes. An exception is SaPIbov2, carried by bovine mastitis isolates, which is 27 kb long and carries a transposon (6). This transposon, which is 12 kb long, contains bap, the gene for a ~240-kDa adhesin that is involved in biofilm formation (5) and has a significant role in bovine mastitis (6). bap is common not only in bovine mastitis S. aureus strains but also in various non-S. aureus staphylococci of veterinary origin (22); although it does not seem to be part of any specific mobile element, it has almost certainly been horizontally transferred. Although the functionality of the SaPIbov2 transposon has not been demonstrated, it is likely to have been responsible for the insertion of bap into SaPIbov2.

Although the overall genetic organization of the SaPIs is largely conserved, there are significant differences in the details, which has led to the development of several individual SaPIs as prototypes for molecular genetic analyses. In addition to SaPI1 (11, 17), we have developed SaPIbov1 (7, 13, 23), the prototype for bovine strains, and SaPI2 (11, 21), the prototype for human toxic shock syndrome, and in this report we describe analysis of a fourth SaPI, SaPIbov2, that has previously been sequenced (25). SaPIbov2 is closely related to SaPIbov1 (7) and uses the same att site, and both are unusual among the SaPIs described thus far in that they show spontaneous integration and excision, similar to the SCCmec elements (9), which leads to a low level of hereditary instability. SaPIbov2 is

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‡ Published ahead of print on 1 June 2007.
included in our set of prototypes, although the closely related SaPIbov1 is better characterized (23), both because we have succeeded in obtaining a stable mutation of SaPIbov2 int but not of SaPIbov1 int and because SaPIbov2 lacks one of the genes that we have recently found to be required for SaPI capsid morphogenesis (24). Our present focus is on the role of SaPIbov2 int in the ERP cycle and on phage specificity in SaPIbov2 induction and transfer. Our results, obtained using SaPIbov2 as a model, suggest three possible modes of SaPI transfer: (i) typical high-frequency SPST accompanying induction of the ERP cycle; (ii) specific recA-independent transfer by phages that do not induce replication, which requires a functional SaPIbov2 int gene in the recipient strain but not in the donor; and (iii) standard, recA-dependent generalized transduction. Additionally, we found that SaPIbov2 can be transferred by several different phages to a variety of non-S. aureus staphylococci.

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions.** Bacterial strains used in these studies are listed in Table 1. Bacteria were grown at 37°C overnight on tryptic soy agar supplemented with antibiotics for plasmid maintenance. Broth cultures were grown aerobically (with shaking at 240 rpm) at 37°C in tryptic soy broth (TSB) without antibiotics.

**DNA methods.** General DNA manipulations were performed by following standard procedures (2, 18). Plasmid DNAs from *Escherichia coli* and staphylococci were purified with a Genelute plasmid mini prep kit (Sigma) used according to the manufacturer’s protocol, except that the staphylococci were lysed by lysozyme (12.5 μg/ml; Sigma) at 37°C for 1 h before plasmid purification. Plasmids were introduced into the staphylococcal strains by electroporation using a previously described method (5). Restriction enzymes were purchased from Roche and were used according to the manufacturer’s instructions. Oligonucleotides were obtained from Invitrogen.

Staphylococcal chromosomal DNA was extracted using a Genelute bacterial genomic DNA kit (Sigma) according to the manufacturer’s protocol, except that the bacterial cells were lysed by lysozyme as described above.

For Southern blot hybridization, agarose gels containing electrophoretically separated DNA fragments were blotted onto nylon membranes (Hybond-N 0.45-μm-pore-size filters; Amersham Life Science) using standard methods (2, 18). Oligonucleotides SaPIbov-6c (5'-CCGGAATTCGTTCATATTAATAATTA AACATTGCTAATC-3') and SaPIbov-6fBgl (5'-GGAGAATTCCTTACATATTTAGGCAC-3') were used to generate the specific SaPIbov2 probe. Labeling of the probes and DNA hybridization were performed according to the protocol supplied with a PCR-DIG DNA-labeling and chemiluminescence detection kit (Roche).

A SaPIbov2 derivative with *tetM* inserted into *bap* was constructed by PCR amplification of the 5' and 3' parts of the *bap* gene, including the promoter region. Oligonucleotides Bap-25mE (5'-CCGGAAATTCCTTACATATTTAGGCAC-3') and SaPIbov-6fBgl (5'-GGAGAATTCCTTACATATTTAGGCAC-3') were used to generate the specific SaPIbov2 probe. Labeling of the probes and DNA hybridization were performed according to the protocol supplied with a PCR-DIG DNA-labeling and chemiluminescence detection kit (Roche).

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this culture in sterile TSB were plated on tryptic soy agar containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (150 μg/ml). White colonies, which did not contain the pMAD plasmid, were tested to confirm replacement by DNA sequencing. Primers were obtained from Invitrogen Life Technologies.

An int clone was constructed by amplifying the int gene from S. aureus c104 with high-fidelity thermophilic DNA polymerase (Dynazyme Ext; Finnzymes) and with primers sip-16mB (5′-CCCAGATGTGCCATCGCAAACACGACATC-G-3′) and sip-17eE (5′-CGGATCTGGTCTTGAATAGTTTGATAGC-3′). The PCR product was cloned into the BamHI/EcoRI sites of pCN51 (4), and the resulting plasmid, pJP33, was transformed by electroporation into S. aureus RN4220. Phase 08a was used to transduce pJP33 from RN4220 to other S. aureus strains (15).

SaPIbov2 excision and circularization were analyzed using primers Ipl-1m and Ipr-2c (excision) and primers Ipl-16cB and Ipr-28m (circularization), as previously described (25).

**Induction of prophages.** Bacteria were grown in TSB to an optical density at 540 nm of 0.4 and induced by adding mitomycin C (MC) (2 μg/ml). Cultures were grown at 32°C with slow shaking (80 rpm). Lysis usually occurred within 3 h. Samples were removed at various time points after phage induction, and standard sodium dodecyl sulfate minilyses were prepared and separated on 0.7% agarose gels, as previously described (11).

Procedures for preparation and analysis of phages lysates, lysogens, and transduction in S. aureus were performed essentially as previously described (15). Experiments were performed at least five times. Similar results were obtained in all cases.

**Real-time quantitative PCR.** Total S. aureus DNA was prepared as described above and analyzed by real-time quantitative PCR using an iCycler machine (Bio-Rad) and the LC-DCNA master SYBR green I mixture (Bio-Rad). The SaPIbov2 DNA was amplified using primers bap-6m and bap-7c (5). The gyrB DNA was amplified as an endogenous control using primers gyr-L (5′-CAACCA TGTAAAACCCACGAGAT-3′) and gyr-U (5′-TTATGTTCTGGCGGCAAATA CA-3′). The level of SaPIbov2 DNA was normalized with respect to gyr DNA. Specificity was confirmed by determining melting curves and by electrophoresis of the final PCR products. In each experiment, all the reactions were performed in triplicate. The relative DNA levels in the different experiments were determined by using the 2^−ΔΔCt method (12).

**RESULTS**

**Phages inducing SaPIbov2 replication and transfer.** In order to enable genetic manipulation, we have introduced selective markers into SaPI genomes. For this study, we introduced by standard allelic replacement the tetM marker into the bap gene of SaPIbov2 in strain c104, resulting in strain JP2025. Note that bap is within a transposon (25) for which we have been unable to demonstrate activity and therefore suggest that it is probably defective and that the inserted tetM gene can be safely used to monitor SaPIbov2 activity. Note that this transposon is the only region of SaPIbov2 that is certainly not involved in SaPI biology, since we have recently demonstrated for SaPIbov1 that the SaPI homologous region is involved in replication and/or transfer (unpublished results).

An important question for understanding SaPI mobility is the relationship between a temperate phage and a SaPI that it induces. This question has several parts: (i) how commonly is a SaPI induced by an endogenous prophage in its native host strain; (ii) how common, in general, are phages that can induce at least one SaPI; (iii) what are the specific mechanisms of SaPI transfer; and (iv) what is the specific phage gene(s) that is involved in induction of the SaPI ERP cycle? In this study, we address primarily the second and third questions. It is already known that phage 80α, which is very closely related to phage 53 of the international typing set (A. Matthews and R. P. Novick, unpublished data) can induce several different SaPIs, including SaPI1, SaPI2, and SaPIbov1, whereas φ11 can induce SaPIbov1 but neither of the other two SaPIs (11, 23).

**Induction by 80α.** In the first part of this study, we surveyed several temperate staphylococcal phages for the ability to induce SaPIbov2, using tetracycline resistance (Tc+) as a marker, starting with 80α. To do this, strain JP2025 was infected with 80α, SaPIbov2::tetM was transduced to RN27, an 80α lysogen, and the resulting strain, JP2130, was induced with MC and tested for induction of the SaPIbov2 ERP cycle. Although the typical SaPI-specific band was not seen on a stained agarose gel containing cellular DNA isolated 90 min after MC induction, a Southern blot of this gel, obtained using a bap-specific probe, revealed dramatic amplification of the SaPIbov2 signal concomitant with the bulk (sheared chromosomal and phage) DNA (Fig. 1). Similar results were obtained using an int-specific probe (not shown). This amplification was confirmed using real-time quantitative PCR to determine the number of SaPIbov2 copies present in the bacteria after induction. In one representative experiment, after 90 min of exposure to MC the relative increase in the amount of DNA for wild-type strain JP2130 was 10.2-fold, whereas the relative increase for Δint strain JP2487 was 6-fold. The resulting lysate showed very-high-frequency SPST, with 5.7 transductants/PFU (Table 2); these results show that SaPIbov2 is strongly induced by 80α. SaPIbov2 does not give rise to the typical SaPI band following induction because it lacks a gene (corresponding to SaPIbov1 orf8) required for the formation of specific small SaPI particles (24). Note that SaPIbov1 with a mutation in orf8 very efficiently packages SaPIbov1 DNA in normal-size phage particles (24), and we assume that the same is true for SaPIbov2. Since RN27 is lysogenic for φ13 as well as for 80α, we repeated the induction experiment with JP2523 (RN4220 80α), which contains no second prophage, and obtained similar results (Fig. 1 and Table 2), except that RN27 seemed to show a greater ability to replicate SaPIbov2 DNA and a higher transduction frequency than the RN4220 derivative, possibly owing to the presence of φ13 in the former.

**Other phages.** The data on phage specificity of SaPI induction are rather limited. In particular, there is very little information on the prevalence of SaPI-mobilizing prophages among uncharacterized clinical strains. To begin an evaluation of the general prevalence of phages inducing SaPIbov2, we examined several of the phages from the international typing set, all of which have recently been sequenced (10), plus several of the typing phage propagating strains, which contain uncharacterized prophages and possibly SaPIs. We constructed lysogens of RN4220 or RN450 and then introduced SaPIbov2::tetM by transduction with 80α. All of these derivative strains were tested for lysogeny with 80α and found to be negative, and they were then tested with MC for the induction of SaPIbov2 replication and transfer. SaPIbov2 replication was tested by preparing whole-cell lysates of the lysogens and propagating strains before and 90 min after MC induction. These lysates were separated on agarose and Southern blotted with an SaPIbov2-specific probe. We interpret the results, shown in Fig. 1, as follows. If there was no clear difference in intensity between the two samples, we concluded that SaPIbov2 replication was not significantly induced by the resident prophage. If the induced sample showed greater intensity than the uninduced sample, we concluded that SaPIbov2 replication was induced and we determined the apparent degree of induction as shown

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in Table 2. For most of the SaPIbov2-containing lysogens, SaPIbov2 replication was undetectable by this method.

Since all phages that have been found to promote SPST are known to be generalized transducing phages (14), we began with the assumption that SaPIbov2 transfer could occur either by SPST or by CGT and that the transfer frequencies would reveal clear differentiation. Instead, as shown in Table 2, there was a wide range of transfer frequencies, and all of the phages that did not induce SaPIbov2 replication transferred the SaPI with frequencies of >10^-6, which is higher than the frequencies usually seen with classical CGT (generally <10^-7). We therefore considered the possibility that in strains where SaPIbov2 replication was not induced, the island was transferred by a mechanism distinct from CGT. To address this possibility, we analyzed the SaPIbov2-phage combinations for the effects of recA (required for CGT) and SaPIbov2 integrase (required for SPST).

**Effect of recA.** To determine whether these results simply represented higher-than-usual frequencies of CGT, we tested for the effects of recA by repeating the transduction experiments with a recA mutant strain, RN981, as a recipient. As shown in Table 2, there was a modest reduction in SPST to recA mutant strains compared to SPST to recA+ lysates. Nevertheless, apparently significant recA-independent transfer was observed with all of the lysates. The frequencies were then compared with the transduction frequencies for a marker known to require recA, an ermB insert in the aureolysin (aur) gene. The transduction of this marker to the recA mutant recipient was below the level of detection (<10^-9 transductants/PU) (Table 3). Even with φ11 (JP2131), however, which does not induce SaPIbov2 replication, SaPIbov2 was readily transferred to recA, with a frequency between 60- and 300-fold lower than the frequency of transfer to recA+ (Tables 2 and 3), suggesting that pure CGT is not seen in this system.

**Effect of integrase.** As expected, and consistent with the SaPI1 results (P. Barry and R. P. Novick, submitted for publication), an integrase mutation (Δint) caused a 10^5- to 10^6-fold reduction in SaPIbov2 transfer to the recA+ recipient for each of the phages tested (Table 2), including the intermediate frequency nonreplicative transfer, as well as standard SPST. As also expected, the Δint mutation blocked excision and circularization, either spontaneously or following MC induction of a lysogen, as revealed by a PCR test for the circular form of SaPIbov2 (Fig. 2). Remarkably, the Δint mutant showed only a modest decrease in replication following MC induction, as revealed by real-time quantitative PCR (see above), suggesting replication in situ.

As also shown in Table 2, transfer of SaPIbov2 Δint to the
that transductants in induced strain.

**TABLE 2. Transduction frequencies for MC-induced SaPIbov2-containing lysogens**

<table>
<thead>
<tr>
<th>Donor strain</th>
<th>SaPIbov2 derivative</th>
<th>Prophage</th>
<th>SaPI replication</th>
<th>Phage titer</th>
<th>SaPI transfer titer (Te⁺ transductants/ml)</th>
<th>SaPI transfer frequency (Te⁺ transductants/PFU)</th>
<th>recA/recA⁺ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>JP2130</td>
<td>Wild type</td>
<td>+ + +</td>
<td>2.1 × 10⁶</td>
<td>1.2 × 10⁹</td>
<td>1.0 × 10⁸</td>
<td>5.7</td>
<td>0.07</td>
</tr>
<tr>
<td>JP2487</td>
<td>Δint</td>
<td>+ +</td>
<td>1.6 × 10⁶</td>
<td>2.4 × 10³</td>
<td>&lt;1</td>
<td>1.5 × 10⁻⁵</td>
<td>&lt;6.2 × 10⁻⁹</td>
</tr>
<tr>
<td>JP2131</td>
<td>Wild type</td>
<td>φ11</td>
<td>4.6 × 10⁶</td>
<td>9.4 × 10⁴</td>
<td>30</td>
<td>2.0 × 10⁻⁶</td>
<td>6.5 × 10⁻⁹</td>
</tr>
<tr>
<td>JP2488</td>
<td>Δint</td>
<td>φ11</td>
<td>8.0 × 10⁴</td>
<td>20</td>
<td>&lt;1</td>
<td>2.5 × 10⁻⁹</td>
<td>&lt;1.2 × 10⁻¹⁰</td>
</tr>
<tr>
<td>JP1642</td>
<td>Wild type</td>
<td>Δint</td>
<td>1.4 × 10⁶</td>
<td>3.0 × 10⁴</td>
<td>7.0 × 10³</td>
<td>2.1 × 10⁻⁵</td>
<td>5.0 × 10⁻⁶</td>
</tr>
<tr>
<td>JP2489</td>
<td>Δint</td>
<td>φ147</td>
<td>1.3 × 10⁵</td>
<td>740</td>
<td>&lt;1</td>
<td>5.7 × 10⁻⁷</td>
<td>&lt;7.7 × 10⁻¹⁰</td>
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<td>JP2525</td>
<td>Wild type</td>
<td>φ55</td>
<td>7.1 × 10⁹</td>
<td>1.9 × 10⁵</td>
<td>30</td>
<td>2.6 × 10⁻⁶</td>
<td>4.2 × 10⁻⁸</td>
</tr>
<tr>
<td>JP2526</td>
<td>Δint</td>
<td>φ55</td>
<td>5.5 × 10⁹</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1.8 × 10⁻⁹</td>
<td>&lt;1.8 × 10⁻⁹</td>
</tr>
<tr>
<td>JP2521</td>
<td>Wild type</td>
<td>φ69</td>
<td>6.3 × 10⁷</td>
<td>7.0 × 10⁴</td>
<td>3.9 × 10³</td>
<td>1.1 × 10⁻³</td>
<td>6.2 × 10⁻⁵</td>
</tr>
<tr>
<td>JP2522</td>
<td>Δint</td>
<td>φ69</td>
<td>8.8 × 10⁷</td>
<td>20</td>
<td>&lt;1</td>
<td>2.2 × 10⁻⁷</td>
<td>&lt;1.1 × 10⁻⁷</td>
</tr>
<tr>
<td>JP2523</td>
<td>Wild type</td>
<td>80α</td>
<td>+ + +</td>
<td>2.4 × 10⁹</td>
<td>9.0 × 10⁷</td>
<td>0.37</td>
<td>0.05</td>
</tr>
<tr>
<td>JP2524</td>
<td>Δint</td>
<td>80α</td>
<td>1.2 × 10⁹</td>
<td>3.5 × 10⁴</td>
<td>&lt;1</td>
<td>2.9 × 10⁻⁴</td>
<td>&lt;8.3 × 10⁻⁹</td>
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<tr>
<td>JP2527</td>
<td>Wild type</td>
<td>φ85</td>
<td>4.8 × 10⁹</td>
<td>1.8 × 10⁵</td>
<td>50</td>
<td>3.7 × 10⁻⁷</td>
<td>1.0 × 10⁻⁸</td>
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<tr>
<td>JP2528</td>
<td>Δint</td>
<td>φ85</td>
<td>6.4 × 10⁹</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1.6 × 10⁻¹⁰</td>
<td>&lt;1.6 × 10⁻¹⁰</td>
</tr>
<tr>
<td>JP2506</td>
<td>Wild type</td>
<td>UC</td>
<td>9.0 × 10⁹</td>
<td>130</td>
<td>&lt;1</td>
<td>1.4 × 10⁻⁸</td>
<td>&lt;1.1 × 10⁻¹⁰</td>
</tr>
<tr>
<td>JP2509</td>
<td>Wild type</td>
<td>UC</td>
<td>+</td>
<td>5.7 × 10⁶</td>
<td>1.2 × 10⁵</td>
<td>2.1 × 10⁻⁴</td>
<td>1.4 × 10⁻⁵</td>
</tr>
<tr>
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<td>Wild type</td>
<td>UC</td>
<td>+ + +</td>
<td>1.0 × 10¹⁰</td>
<td>8.2 × 10⁵</td>
<td>8.2 × 10⁻⁵</td>
<td>2.6 × 10⁻⁵</td>
</tr>
</tbody>
</table>

*a* The data are data from a representative experiment.  
*b* MC, uncharacterized.  
*c* Number of phage particles/ml of induced culture, using RN4220 as the recipient strain.  
*d* Number of SaPIbov2 particles/ml of induced culture, using RN4220 or RN981 (recA mutant) as the recipient strain.  
*e* SaPIbov2 transfer titer divided by phage titer.

**TABLE 3. Transfer of SaPIbov2-tetM and chromosomal aur·ermB**

<table>
<thead>
<tr>
<th>Prophage</th>
<th>SaPIbov2</th>
<th>MC-induced phage titer</th>
<th>SaPIbov2 titer (Te⁺)</th>
<th>aur·ermB titer (ErmB)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>RN4220</td>
<td>RN981</td>
</tr>
<tr>
<td>80α</td>
<td>Wild type</td>
<td>1.4 × 10⁸</td>
<td>1.3 × 10⁸</td>
<td>6.8 × 10⁸</td>
</tr>
<tr>
<td>80α</td>
<td>Δint</td>
<td>3.2 × 10⁸</td>
<td>1.4 × 10⁵</td>
<td>0</td>
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<tr>
<td>φ11</td>
<td>Wild type</td>
<td>3.2 × 10⁹</td>
<td>5.2 × 10⁵</td>
<td>90</td>
</tr>
<tr>
<td>φ11</td>
<td>Δint</td>
<td>8.3 × 10⁹</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>φ147</td>
<td>Wild type</td>
<td>4.4 × 10⁹</td>
<td>2.6 × 10³</td>
<td>1.9 × 10³</td>
</tr>
<tr>
<td>φ147</td>
<td>Δint</td>
<td>6.3 × 10⁹</td>
<td>560</td>
<td>0</td>
</tr>
</tbody>
</table>

*a* The data are data from a representative experiment.  
*b* Number of phage particles/ml of induced culture, using RN4220 as the recipient strain.  
*c* Number of SaPIbov2 particles/ml of induced culture, using RN4220 or RN981 (recA mutant) as the recipient strain.  
*d* Number of ErmB-resistant transductants/ml of induced culture, using RN4220 or RN981 (recA mutant) as the recipient strain.

*recA* mutant recipient could not be detected with any of the phages tested, indicating that *int* is required for even the low-level residual transfer to the *recA* mutant recipient observed with phages φ11, φ55, and φ85 and therefore that pure CGT can account for only a fraction of the SaPIbov2 transductants obtained with nonmobilizing phages. Since *int* is required for SaPI integration into the recipient chromosome, we suggest that one possible explanation for these results is that phages unable to induce the SaPIbov2 ERP cycle capsidate and transfer chromosomal fragments containing the SaPI as they would for any other chromosomal locus. In a *recA* mutant recipient, any gene thus transferred would be lost; with a fragment containing SaPIbov2, however, the integrase would be expressed and could catalyze excision of the element and insertion into its specific *attC* site. Alternatively, spontaneously excised SaPIbov2 circular monomers (25) could be the substrate for encapsidation and, following transfer, could circularize and integrate. Note that in the first mechanism proposed *int* would be necessary only in the recipient strain, while in the second *int* would be necessary in both the donor and recipient strains.

**Int complementation.** To distinguish between these two possibilities, we cloned SaPIbov2 *int* into pCN51, generating plas-
mid pJP433, in which int is driven by a Cd-inducible promoter (4). As this promoter has considerable basal activity, cadmium induction is usually not required. As shown in Table 4, when a recA mutant recipient was used, int was required in the recipient but not in the donor for recA-independent SaPIbov2 transfer by phages such as \( \phi 11 \) and \( \phi 147 \) that do not induce the ERP cycle and do not induce SaPIbov2 excision and circularization (not shown). This result clearly supports the model shown in Fig. 3 and effectively rules out the possibility that excised circular molecules in the donor represent the substrate for this transfer. This mode of low-frequency transfer is referred to replication-independent SaPI transfer. It was noted that the frequencies of SaPIbov2 transfer by this intermediate mechanism vary over a very wide range (\( 10^{-6} \) to \( 10^{-3} \)) with the set of generalized transducing phages studied, as shown in Table 2. This could simply reflect differences in the intrinsic transducing activity of these phages, a possibility that has not been critically analyzed.

**Transspecies transfer of SaPIbov2.** It is generally accepted that phage-mediated transduction is the most important mechanism of horizontal gene transfer in the staphylococci. Accordingly, accessory genes occurring in different staphylococcal strains and species are usually assumed to have been acquired by transduction. A case in point is bap, which we have previously identified in a variety of different staphylococcal species, including *S. epidermidis*, *S. chromogenes*, *S. xylosus*, *S. simulans*, and *S. hyicus* (22). We noted that bap is not present in the sequenced genomes of any of the staphylococci, including *S. epidermidis*, indicating that it is a variable gene. Given the rather common occurrence of *S. aureus* phages capable of mobilizing SaPIbov2, it seemed possible that transspecific transduction of the island might occur, which would represent a theoretical demonstration of transspecific gene transfer. As shown in Table 5, we were able to demonstrate transduction of SaPIbov2 to *S. xylosus* and to several *S. epidermidis* strains at frequencies that were about 0.1% of the frequency of transduction to RN4220. Interestingly, it was not possible to transfer the SaPIbov2 \( \Delta int \) island to the coagulase-negative staphylococci, which indicates that the transfer was int mediated. In

![Figure 2](image1.png)

**FIG. 2.** Precise excision and circularization of SaPIbov2 mediated by the Int protein. (A) Detection of Int mediated SaPIbov2 excision and formation of \( att_\mu \). DNA from MC-induced strains were extracted (at 0 and 90 min) and PCR amplified using specific primers Ipl-1m and Ipr-2c recognizing the flanking sequence of the SaPIbov2 island. (B) Detection of int-mediated SaPIbov2 circularization. Samples obtained as previously described were PCR amplified with a pair of primers used divergently at both termini of the SaPIbov2 island (primers Ipl-16cB and Ipr-28m). JP2130 is a wild-type strain, and JP2487 is an int mutant strain.

![Figure 3](image2.png)

**FIG. 3.** Int-mediated mechanism involved in SaPIbov2 transfer.

**TABLE 4.** Complementation of the SaPIbov2 \( \Delta int \) mutant

<table>
<thead>
<tr>
<th>SaPIbov2 ( \Delta int ) strain</th>
<th>Prophage</th>
<th>Plasmid</th>
<th>Recipient strain titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor strain</td>
<td></td>
<td></td>
<td>Empty pCN51 pCN51-int</td>
</tr>
<tr>
<td>JP247</td>
<td>( \phi 11 )</td>
<td>pCN51</td>
<td>0 0 580</td>
</tr>
<tr>
<td>JP248</td>
<td>( \phi 11 )</td>
<td>pCN51-int</td>
<td>0 0 110</td>
</tr>
<tr>
<td>JP249</td>
<td>( \phi 147 )</td>
<td>pCN51</td>
<td>0 0 590</td>
</tr>
<tr>
<td>JP250</td>
<td>( \phi 147 )</td>
<td>pCN51-int</td>
<td>0 0 790</td>
</tr>
</tbody>
</table>

* The data are data from a representative experiment.

* Number of SaPIbov2 particles/ml of induced culture, using RN981 \( (\text{recA mutant}) \) as the recipient strain.
S. aureus, the 18-bp SaPIbov2 att gene is at the 3′ end of the GMP synthase (gmpS) gene (25). To determine whether SaPIbov2 integrates at the same site in the S. epidermidis or S. xylosus chromosome, JP1522 (a SaPIbov2-containing S. xylosus strain) and JP2632, 2633, 2557, and 2558 (SaPIbov2-containing S. epidermidis strains) were analyzed by PCR using primers Ipr-13mP (specific for SaPIbov2) and Ipr-2c (specific for the gmpS gene [25]), designed to amplify the right junction fragment of SaPIbov2. In all these strains, a 0.7-kb band comprising the right junction of SaPIbov2 and having the same mobility as the corresponding product from RN4220 containing SaPIbov2 (Fig. 4) was obtained. Sequence analysis of these PCR products confirmed the site-specific localization of SaPIbov2 in the att site of the S. xylosus and S. epidermidis strains analyzed. It is additionally interesting that a BLAST search for the SaPIbov2 att site revealed that each of the S. epidermidis sequenced genomes and the S. saprophyticus genome contain one copy of the site, at the 3′ end of the gmpS gene, but no other copy, indicating that the site is unoccupied. It is also unoccupied in all of the sequenced S. aureus genomes (with the exception of the genome of RF122 [the native host for SaPIbov1]) and in all 43 toxic shock strains that we have analyzed independently (21).

**DISCUSSION**

In this study, we demonstrated mobilization and high-frequency transfer of a fourth SaPI prototype, SaPIbov2, the largest of the known elements in this class, the second such element from a veterinary source, and a close relative of the first element, SaPIbov1. SaPIbov2 and SaPIbov1 both use the same chromosomal att site, at the 3′ end of gmpS, a site that is not used by any of the other known SaPIs, all of which are from human isolates, including those in a set of 43 toxic shock syndrome isolates that we have recently characterized (21). SaPIbov2 shows the known classical SaPI ERP cycle upon SOS induction of a coresident 80α prophage, except that the typical SaPI-specific monomer band is not seen in an agarose gel. This is because SaPIbov2 completely lacks one of the genes, corresponding to SaPIbov1 ORF8, that we have shown to be required for the formation of the typical small-headed SaPI particles (24). Since this band is derived from disruption of mature SaPI particles during the preparation of DNA samples for electrophoretic analysis (24), this is not surprising because the 27-kb SaPIbov2 genome is too large to be encapsidated in such particles. This finding raises a number of interesting questions. First, since SaPIbov2 is about 12 kb larger than any of the 16 other known SaPIs (16), owing to the insertion of a 12-kb transposon, did it “lose” the ORF8 homolog after acquisition of the transposon, or did the transposon insert into a SaPI genome that was already missing this gene? Since SaPIbov2 is stably maintained as a chromosomal element, selection for transferability could account for the evolutionary loss of the ORF8 homolog. Alternatively, since at least two of the known SaPIs, SaPI5 and SaPIbw2, are missing all or part of the capsule morphogenetic system (16), the transposon could have inserted into an element lacking this system. Second, since SaPIbov2 is transferred at the same high frequency as other SaPIs and possesses the typical phage terminase small subunit required for SaPI-specific packaging, it must package its DNA in standard phage heads, as an ORF8 mutant of SaPIbov1 does (24). What, then, is the biological advantage of SaPI-specific capsid morphogenesis? One possibility is that since the small-headed SaPI particles can encapsidate only about one-third of the inducing phage genome, coinfection with a SaPI-containing particle and a small-headed particle containing phage DNA will result only in SaPI transfer. Third, why is there no SaPIbov2-specific band in lysates of cells in which SaPIbov2 is replicating? Presumably this material would consist of ~40 kb, representing about 1.5 SaPIbov2 genomes, and would comigrate with the bulk DNA in the gel. Experiments to demonstrate it are in progress.

Among the known phages tested, only 80α could induce the SaPIbov2 ERP cycle. Examination of several uncharacterized strains to which SaPIbov2 had been transferred suggested that at least one strain, the propagating strain for typing phage X2, contained a prophage that could induce the SaPIbov2 ERP cycle. Experiments to characterize this phage are in progress. recA-independent transfer was observed with all five of the noninducing phages and with at least one of the uncharacterized phages carried by PS77, whereas recA-independent transfer was not detectable with a standard chromosomal gene. This suggested that there was a SaPI-specific transfer mechanism that was not dependent on the replication-induced formation of the multimeric substrates required for the standard headful packaging mechanism. One possibility was that the spontaneously excised circular SaPIbov2 monomers, previously shown to be present (25), could have been encapsidated and transferred. This was unlikely because such monomers would be too

![FIG. 4. Integration-specific PCR: PCR analysis using primers Ipr-13mP and Ipr-2c to test the site-specific integration of the SaPIbov2 island in the coagulase-negative staphylococci mediated by Int activity. JP1522 is an S. xylosus SaPIbov2-positive strain, JP2632, JP2633, JP2557, and JP2558 are S. epidermidis SaPIbov2-positive strains, and JP2130 is an S. aureus positive control.](https://example.com/screenshot)
small to fill a phage head and, therefore, some secondary mechanism, such as recombination between two monomeric circles, would have been required. A second possibility was that phage-genome-size chromosomal fragments containing the intact SaPIbov2 genome were generated by the usual mechanism for the formation of generalized transducing fragments and that following transfer, SaPIbov2 was excised, circularized, and inserted into its attC site by the SaPIbov2-encoded integrase, as shown in Fig. 3. In the first mechanism, a functional int (and possibly also a functional recA) would have to be present in the donor strain, whereas in the second scenario, int would be required only in the recipient. Using an int-defective mutant of SaPIbov2, we demonstrated that int was required for replication-independent transfer as well as for classical SPST. The key finding was that a cloned int was required in the recipient but not in the donor for the replication-independent transfer of SaPIbov2, strongly supporting the second of the possibilities described above. We note, incidentally, that int is predictably required in both the donor and the recipient for classical SPST (P. Barry and R. P. Novick, submitted); an experiment to confirm this with SaPIbov2 could not be interpreted owing to the occurrence of SaPI-plasmid recombinants, and correction of this problem is currently in progress. We note also that the replication-independent transfer of SaPIbov2 is decreased to a greater or lesser extent with a recA mutant recipient, suggesting that CGT accounts for some of the transductants and therefore that the int-dependent mechanism is less than 100% efficient. We have no explanation at present for the apparently different frequencies of recA- and int-dependent transfer observed with different phages (Table 2). The three different SaPIbov2 transfer mechanisms are listed in Table 6.

In addition to studies with S. aureus, we investigated SaPIbov2 transfer to several non- S. aureus staphylococci with particular reference to the possible source of the SaPIbov2-carried bap. Bap is a very large adhesin involved in biofilm formation and contributes significantly to bovine mastitis. Its presence within a well-defined transposon in SaPIbov2 suggests strongly that it was acquired by SaPIbov2 via transposition. Since bap has been demonstrated in non- S. aureus staphylococci (22), it was possible that one such species was the source of the SaPIbov2 ortholog. Although bap was not contained either within a SaPI or within a transposon in any of the species that were examined (22), we demonstrated in this study that SaPIbov2 could be readily transferred to each of these species, suggesting at least the possibility that intergeneric SaPI transfer may have been involved in the acquisition by S. aureus of the bap determinant. It remains to be determined, however, whether any of these species contains a prophage capable of transferring SaPIbov2 or other genetic elements to S. aureus.

ACKNOWLEDGMENTS

We express our gratitude to J. Pelletier for providing S. aureus phages and their propagating strains. This work was supported by grant BIO2005-08399-C02-02 from the Comisión Interministerial de Ciencia y Tecnología (C.I.C.Y.T.), by grants from the Cardenal Herrera-CEU University, from the Consellería de Agricultura, Pesca e Alimentación (CAPIA), and from the Generalitat Valenciana (ACOMP06/235) to J.R.P., and by NIH grant R01-AI-22159 to R.P.N. Fellowship support for Carles Úbeda from CAPIA and for Elisa Maiques from the Cardenal Herrera-CEU University is gratefully acknowledged.

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