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SaPI operon I is required for SaPI packaging and is controlled by LexA

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Summary

Transfer of *Staphylococcus aureus* pathogenicity islands (SaPIs) is directly controlled by the cellular repressor LexA. We have found that transcription of the SaPIbov1 operon I is repressed by LexA and is therefore SOS-induced. Two copies of the LexA binding site consensus (Cheo box) are present in the 5′ region of this operon, at the same location in all of 15 different SaPIs analysed. Both of these boxes bind LexA protein. Furthermore, replacement of the chromosomal *lexA* with a non-cleavable mutant LexA (G94E) greatly diminished expression of SaPIbov1 operon I and differentially reduced the production of SaPI transducing particles in comparison with the production of plaque-forming particles. In concordance with this finding, deletion of operon I blocked the formation of SaPI transducing particles but had no effect on replication of the island. Operon I contains a gene encoding a homologue of the phage terminase small subunit plus two other genes that direct the assembly of the small sized SaPIbov1 capsids. Interestingly, mutations affecting the latter two genes were not defective in SaPI transfer, but rather encapsidated the island in full-sized phage heads, which would have to contain a multimeric SaPI genome.

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Introduction

A remarkable feature of bacterial pathogenesis is that the majority of bacterial toxins are encoded by mobile genetic elements (MGEs), including prophages, plasmids transposons and pathogenicity islands (PTIs) (Novick, 2003). This raises a number of interesting questions, including that of what may have been the evolutionary forces responsible for this association, and of what may be its advantage for either the bacterial host or the MGE itself. If one accepts the notion that MGEs are basically selfish genetic elements, it can be argued that they carry toxin genes in order to pay their metabolic rent, so to speak. This raises the related question of what are the selective advantages of toxins for the host bacterium – a question that has a variety of answers depending on the particular toxin, and is not addressed here. More to the point of this report is the fact that most, or all lamboid phages, which maintain the prophage state by repression, are activated by the SOS response to initiate lytic growth via RecA-induced autocleavage of their repressors (Little, 1993). Perhaps they are keyed into the SOS response so as to escape from a lethally damaged host by producing infective particles.

Also related with the SOS response is the family of phage-related 14–17 kb staphylococcal pathogenicity islands, the *Staphylococcus aureus* pathogencity islands (SaPIs), which occupy specific chromosomal sites and are induced to excise and replicate by certain temperate phages (Novick *et al*., 2001; Novick, 2003). The SaPIs were initially identified as repositories of superantigen genes, especially *tst* [encoding toxic shock syndrome toxin-1 (TSST-1)] (Lindsay *et al*., 1998), and most, but not all of the SaPIs carry genes encoding superantigens or other products that specifically impact the host cell's phenotype. Staphylococci almost always carry one or more prophages and it has recently become clear that the vast majority also carry one or more SaPIs (Novick and Subedi, 2007). Sequences are available for 15 of these (Novick and Subedi, 2007), some determined explicitly, others retrieved from the sequenced genomes, of which nine are presently available.

Not only are the SaPIs induced to excise and replicate by temperate phages, but they also induce the phage to produce special small capsids commensurate with their genome sizes, into which they are encapsidated (Ruzin *et al*., 2001; Úbeda *et al*., 2005), resulting in exceptionally high transfer frequencies, so that their transferability, like that of prophages, is clearly a strongly selected characteristic of their biology. The SaPIs are induced by a matching temperate phage whether by superinfection of a phage-sensitive SaPI-carrying host, or by spontaneous or SOS induction of a resident prophage. Although all of these processes probably occur naturally, we are presently concerned primarily with the third, SOS induction, and secondarily with the question of whether spontaneous prophage induction involves the SOS system. The SOS response, initiated by DNA damage, involves activation of RecA, which in turn induces autocleavage of the cellular repressor LexA. LexA binds to specific 5′ regulatory motifs (LexA boxes) in the SOS-regulated genes, including *recA,* and is therefore the primary SOS repressor. LexA is widespread among bacteria and is present in most phylogenetic groups, for which different monophyletic LexA-binding motifs have been described. For example, the consensus LexA binding motif in *Escherichia coli* is $CTGTN_8ACAG$ (Walker, 1984) whereas that in Gram-positives (Cheo box) is $GAACN_4GTTC$ (Cheo *et al*., 1991). The direct involvement of LexA in SOS induction of prophages such as coliphage 186 (Lamont *et al*., 1989) and the CTX phage of *Vibrio cholerae* (Quinones *et al*., 2005) but not of lambdoid prophages indicates that whereas LexA is an essential component of the bacterial SOS response, it is not a universal direct component of the SOS response with respect to prophage induction, other than being a repressor of *recA*.

Here, we report that LexA is directly involved in SOS induction of the SaPIs. We find that an operon essential for SaPI packaging, but not for replication, is directly regulated by LexA. This operon encodes a homologue of the phage terminase small subunit, required for the specific packaging of SaPI DNA, plus other proteins required for the formation of SaPI-specific small capsids. LexA binds to two LexA boxes in the promoter region of operon I and blocks transcription. SOS induction relieves this repression, as is typical for LexA-repressed genes, enabling the formation of the SaPI-specific small capsids and the encapsidation of SaPI monomers.

Results

Elimination of LexA cleavage differentially affects SaPI-specific transfer (SPST) in comparison to phage growth

In this and other articles from our two laboratories, we use SPST to refer to phage-induced formation of SaPI-specific transducing particles, as opposed to CGT, for classical generalized transduction. To test for the possibility that LexA is involved in SaPI induction, we initially tested two pairs of SaPIbov1-containing strains, lysogenic for either 80α or φ11. One strain of each pair had wt *lexA*, the other had a derivative of *S. aureus. lexA* encoding an amino acid replacement, G94E, previously shown to prevent cleavage of the homologous *E. coli* LexA (Maiques *et al*., 2006). These strains were induced with mitomycin C, the supernatants were collected and SaPI transductions, as well as phage titres, were determined. As shown in Table 1, the results with the two phages were inconsistent. With 80α, the non-cleavable LexA caused an approximately 5000-fold reduction in phage titre and a similar reduction in SPST, whereas with φ11 there was only a slight reduction in phage titre but a considerably greater reduction in SPST with the mutant LexA. In an attempt to obtain a clearer test for the possible differential effects of LexA on phage versus SaPI induction, we used φ69, which we have shown previously to induce the excision–replication–packaging (ERP) cycle for SaPIbov2 (Maiques *et al*., unpubl. results) and we have found that it acts similarly for several other SaPIs, including SaPI1, SaPI3, SaPIbov1 and SaPIn1 (not shown). Accordingly, in Table 1 are shown the results of similar experiments with φ69 lysogens containing each of these SaPIs. Here, the results were much clearer: the defect in LexA cleavage had a considerably greater effect on SPST than on phage growth for all of the SaPIs tested. Note also that all of the strains lysed, and with the exception of 80α, only a modest reduction in phage titres was caused by the LexA defect, indicating that LexA cleavage is not very important for SOS-induced growth of either φ11 or φ69, although it is quite important for 80α.

Identification and testing of LexA binding sites

The above results led us to examine the SaPI genomes for possible LexA binding sites. As *Bacillus subtilis* LexA is highly similar to *S. aureus* LexA, it was not surprising that the consensus *lexA* operator sequence, 5′-$GAACN_4GTTC$-3′ of *B. subtilis* and other Gram-positive bacteria is closely related to that of *S. aureus* (Bisognano *et al*., 2004). Starting with the SaPIbov1 sequence, we identified two sites that matched the consensus LexA binding site for Gram-positive bacteria (Cheo boxes, Cheo *et al*., 1991), 63 and 176 bp upstream of the SaPIbov1 open reading frame (ORF) 10 coding sequence, as shown in Fig. 1A, but not elsewhere in the SaPIbov1 genome. We demonstrated by reverse transcription polymerase chain reaction (RT-PCR) that ORF10 is the first gene in a 6-gene operon (data not shown), which is highly conserved in at least 12 of the 15 SaPIs for which sequences are available, and which contains these same

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Table 1. Effect of non-cleavable LexA on phage induction and SaPI transduction.[a]

Donor strain	φ	SaPI	SaPI (titre)[b]	Phage titre[c]	Transduction (titre)[d]	% transduction[e]
JP44	80α	SaPIbov1	1.5×10^9	5.1×10^9	0.294	–
JP85 (JP44 LexA G94E)	80α	SaPIbov1	2.3×10^5	1.0×10^6	0.23	78%
JP47	φ11	SaPIbov1	5.8×10^7	9.7×10^6	5.98	–
JP1879 (JP47 LexA G94E)	φ11	SaPIbov1	1.3×10^6	2.7×10^6	0.48	8.0%
JP2897	φ69	SaPI1	3.0×10^4	9.3×10^5	0.032	–
JP2898 (JP2897 LexA G94E)	φ69	SaPI1	10	6.2×10^4	1.6×10^{-4}	0.5%
JP2901	φ69	SaPI3	4.4×10^2	3.1×10^7	1.4×10^{-5}	–
JP2902 (JP2901 LexA G94E)	φ69	SaPI3	< 1	6.4×10^6	$< 1.5 \times 10^{-7}$	< 0.01%
JP2899	φ69	SaPIn1	8.0×10^3	1.6×10^7	5.0×10^{-4}	–
JP2900 (JP2899 LexA G94E)	φ69	SaPIn1	17	2.4×10^6	7.0×10^{-6}	1.4%
JP2895	φ69	SaPIbov2	1.1×10^5	4.4×10^7	2.5×10^{-3}	–
JP2896 (JP2895 LexA G94E)	φ69	SaPIbov2	110	1.6×10^7	6.8×10^{-6}	0.272%
JP1821	φ11	SaPIbov1 ΔORF13	1.1×10^6	2.6×10^7	0.042	–
JP2886 (JP1821 LexA G94E)	φ11	SaPIbov1 ΔORF13	1.0×10^3	4×10^6	2.5×10^{-4}	0.6%

a. The table shows results from a representative experiment.
b. No. transductants × ml of induced culture, using RN4220 as recipient strain.
c. No. of phage plaques × ml of induced culture, using RN4220 as recipient strain.
d. Transduction titre was defined as SaPI titre/phage titre.
e. Percentage of transductants obtained with the LexA mutants strains compared with their corresponding wt strains.

two LexA boxes at the same relative locations (Fig. 1B). LexA binding to the two putative Cheo boxes was then demonstrated by an electrophoretic gel retardation assay using purified *B. subtilis* LexA protein, whose DNA binding domain is 77% identical to that of *S. aureus* LexA, and using separate DNA fragments for each of the two putative LexA binding sites as probes. As shown in Fig. 1C, LexA-specific retardation was seen with both probes. This retardation was fully competed by an excess of unlabelled probe DNA but not by unrelated DNA. Moreover, the LexA protein was unable to bind to mutant derivative probes presenting alterations in the Cheo box sequence, further confirming the LexA binding location in the SaPIbov1 ORF10 promoter region (Fig. 1C).

The functionality of these LexA binding sites was confirmed by transcriptional analysis of operon I, using quantitative RT-PCR, with RNA from strains JP45 (wt) and JP1847 (non-cleavable LexA, G94E). As shown in Fig. 2, the expression of ORFs 5 and 9 were induced by mitomycin C in the wild-type strain but not in the LexA G94E mutant.

Site of LexA regulation in the SaPI ERP cycle

A priori, LexA could regulate any stage of the SaPI induction cycle. However, because LexA represses operon I, it is likely to be this repression that is responsible for the observed effect of non-cleavable LexA on SPST frequency. To test the function of the LexA-regulated SaPI operon, we have deleted the entire operon I and have introduced in-frame deletions into each of the operon I ORFs. Either deletion of the entire operon (data not shown) or the in-frame deletion in ORF5 abolished SPST (Table 2), whereas mutations affecting the other five

Table 2. Effect of SaPIbov1 *ter* mutation in SPST by different prophages.[a]

			Recipient strains[b]		
Donor strain	Phage	SaPI	RN4220	RN981 (*rec*A⁻)	Phage titre[c]
JP47	φ11	SaPIbov1	6.6×10^7	2.7×10^7	1.5×10^7
JP248	φ11	SaPIbov1 *Δter*	2.8×10^4	2×10^4	3.4×10^7
JP3075	φ80α	SaPIbov1	8.5×10^9	5.1×10^9	2.6×10^9
JP3076	φ80α	SaPIbov1 *Δter*	1.6×10^6	1×10^6	4.4×10^9
JP3079	φ55	SaPIbov1	6×10^4	2.2×10^4	2×10^7
JP3080	φ55	SaPIbov1 *Δter*	20	10	3×10^7
JP3081	φ85	SaPIbov1	4×10^6	1.7×10^6	6.2×10^9
JP3082	φ85	SaPIbov1 *Δter*	140	40	6.5×10^9

a. The table shows results from a representative experiment.
b. No. transductants × ml of induced culture, using RN4220 or RN981 (*rec*A mutant) as recipient strain.
c. No. of phage plaques × ml of induced culture, using RN4220 as recipient strain.

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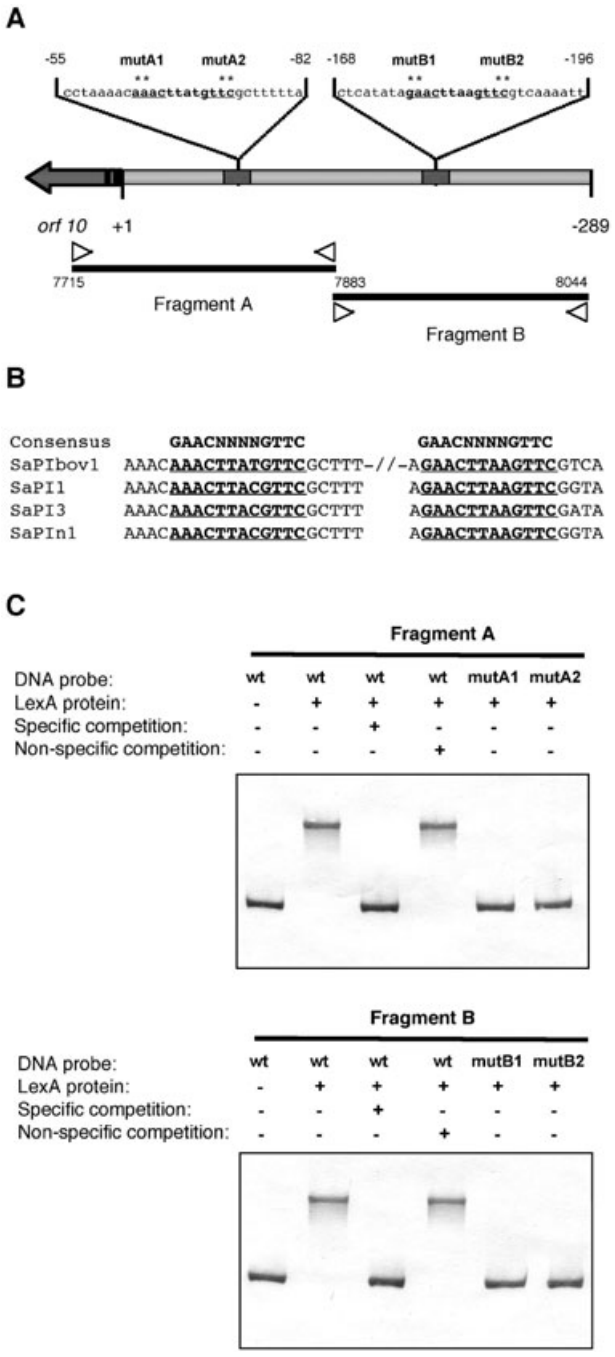

Fig. 1. A. Diagram representing the fragments used in electrophoretic mobility shift assays (EMSA). Arrows indicate the oligonucleotide location. LexA binding sites are shown. The position of base pair substitution in the mutant promoter probes is also indicated by asterisks. In the mutA1 and mutB1 probes, the nucleotides of the wild-type sequence were changed by TT. In the mutA2 and mutB2 probes, they were replaced by AA. Fragments were obtained from the SaPIbov1 *orf*10 promoter by PCR amplification using a DIG-end labelled oligonucleotides. +1: protein start.
B. Alignment of part of the SaPIbov1-operon I sequence, containing the two Cheo boxes (underlined), with other SaPIs sequences. The DNA sequence of SaPIbov1 operon I is compared with the sequences of SaPI1, SaPI3 and SaPIn1.
C. Binding of LexA to SaPIbov1 operon I promoter. Mobility of the wild type (wt) or mutant (mut) DIG-labelled DNA probes in the presence of 100 nM purified *B. subtillis* LexA protein is indicated on the top. In competition assay, 500-fold excesses of specific- or non-specific unlabelled DNA fragments were added.

ORFs did not (data not shown). Because the predicted ORF5 product is highly similar to the phage terminase small subunit, and is well conserved among all of the known SaPIs (Novick and Subedi, 2007), it is suggested that the ORF5 product, hereafter referred to as Ter, is required for SaPI encapsidation. This suggestion was confirmed by an examination of the fate of SaPIbov1 DNA following SOS induction of strains containing SaPIbov1 operon I mutants. In this experiment, as shown in Table S1 (*Supplementary material*), SaPIbov1 DNA was amplified to essentially the same degree with any of the operon I mutants as with the wt island, although the characteristic SaPI band, seen in cell lysates of phage-infected SaPI-containing cells and corresponding to a monomer-sized SaPI genome, could not be detected in the *ter*$^-$ (ΔORF5) strain, or, incidentally, in either the ΔORF8 or the ΔORF9 strain (Fig. 3). With the latter two mutants, however, SPST was unaffected; their role in SPST is addressed below. This result confirms that *ter* is required for SaPIbov1 encapsidation into SaPI-specific small phage heads but not for replication of the island. Therefore, it is predicted that blockage of SOS induction of *ter* expression by non-cleavable LexA diminishes SPST frequency by interfering with encapsidation. If this inference is correct and if there is no other LexA-repressed SaPI promoter, then it would also be predicted that the consequences of *ter* inactivation would be the same in a wt host as in a strain with non-cleavable LexA. Tests of this prediction revealed the somewhat unexpected result

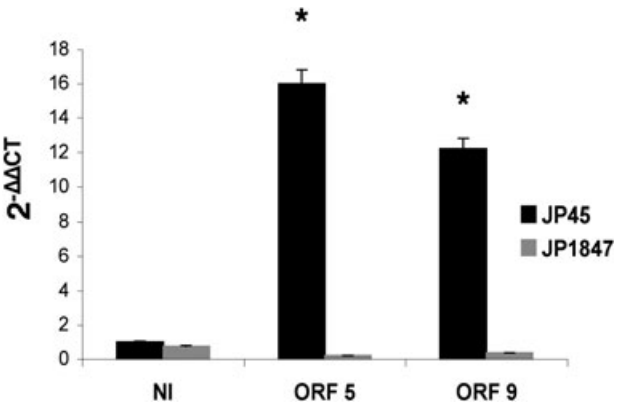

Fig. 2. Real-time quantification of ORF5 and ORF9 expression on *S. aureus* wild-type strain JP45 and its corresponding LexA mutant JP1847 (JP45 LexA G94E), 60 min after of SOS (mitomycin C) induction. NI, not induced. Asterisks denote significance ($P < 0.05$).

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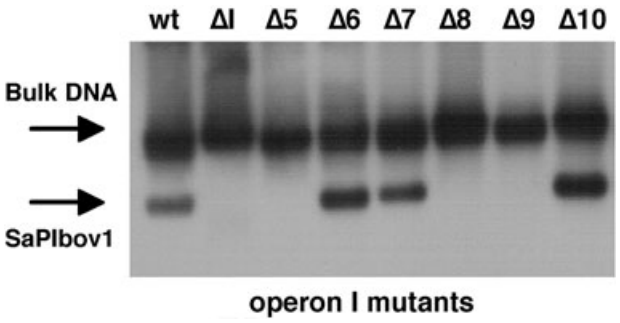

Fig. 3. Role of the operon I genes in the production of SaPIbov1 monomers. Bacterial cultures of 80α lysogens containing the different SaPIbov1 mutants were exposed to mitomycin C, then incubated in broth at 32°C. Samples were removed at 90 min and used to prepare minilysates. Lysates were separated by agarose gel electrophoresis and transferred. Southern blot hybridization pattern of the samples obtained, hybridized overnight with a SaPIbov1-specific probe, are shown. Similar results were obtained using ϕ11 lysogens (data not shown).

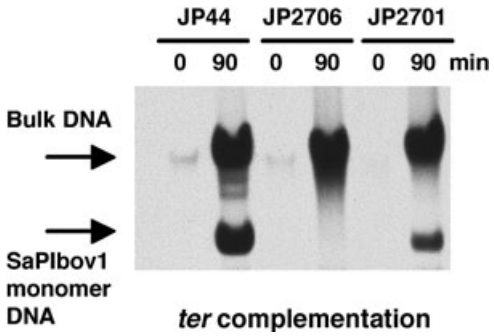

Fig. 5. Complementation studies with the *ter* gene of the SaPIbov1 ORF5 mutant. JP44: SaPIbov1 wt; JP2706: SaPIbov1 ΔORF5 pCN51 (empty vector); JP2701: SaPIbov1 ΔORF5 pJP368.

that the yield of SaPIbov1 Δ*ter* DNA was somewhat reduced in the LexA non-cleavable strain in comparison to the LexA$^+$ strain, but the yield of ϕ11 DNA was reduced to an even greater extent (Fig. 4A). This suggests that the overall replication responses of both SaPI and phage to SOS induction are reduced in parallel, even though the final phage titre is reduced only slightly in the LexA non-cleavable strain (Table 1) – suggesting that the LexA defect slows down phage maturation but does not greatly affect the final outcome. To confirm that replication of SaPIbov1 DNA is independent of LexA, we made use of a recent finding that an inactivating mutation of *stlB* (ORF20) enables replication of SaPIbov1 in the absence of phage (Úbeda *et al.* manuscript in preparation). As shown in Fig. 4B, with this mutant, MC induction (in a non-lysogen) caused a moderate inhibition of SaPIbov1 replication even though operon I is SOS inducible. These results confirm the prediction that the only direct effect of LexA is on operon I expression and therefore that the only LexA-sensitive step in the SaPI ERP cycle is operon I-mediated encapsidation.

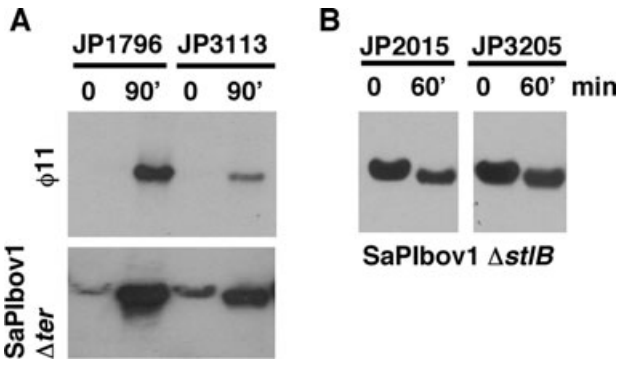

Fig. 4. Role of LexA in SaPI replication. Bacterial cultures of JP1796 (SaPIbov1 *tst*::*tet*M Δ ORF5, ϕ11), its derivative LexA mutant JP3113 (A), or JP2015 (SaPIbov1 *tst*::*tet*M ΔORF20) and its derivative LexA mutant JP3205 (B) were exposed to mitomycin C, then incubated in broth at 32°C. Samples were removed at the indicated time points and used to prepare minilysates. Lysates were separated by agarose gel electrophoresis and transferred. Southern blot hybridization pattern of the samples obtained, hybridized overnight with a SaPIbov1- or ϕ11-specific probe, are shown.

Complementation

To confirm that the observed effects of the *Δter* mutant on SPST were specific for the *ter* gene, we cloned *ter* under the control of a cadmium inducible promoter in pCN51 and transferred the resulting plasmid (pJP368) to JP1747, an 80α lysogen containing SaPIbov1*Δter.* As shown in Fig. 5, a SaPI band was seen following MC induction of 80α in this strain, but not with the vector alone. pJP308 also restored high frequency SPST to SaPIbov1*Δter* (not shown), confirming that the *Δter* mutation was fully responsible for the phenotype of the mutant.

Generality of ter *function*

Finally, we have tested for the generality of Ter function, by comparing the SPST frequencies for SaPIbov1 and its *Δter* derivative with characterized prophages from four different strains. As can be seen in Table 2, lack of the Ter protein caused an approximately 1000-fold decrease in SPST for three of the strains; for the fourth, at least a 40 000-fold decrease, which clearly demonstrate the important role of Ter in SaPI transfer, independently of the host prophage.

Encapsidation specificity: operon I ORFs 6–10

The failure of SaPIbov1 mutants defective in either ORF8 or 9 to produce a SaPI band in DNA blots coupled with high SPST frequencies suggested that these genes are required for the formation of small capsids but are not

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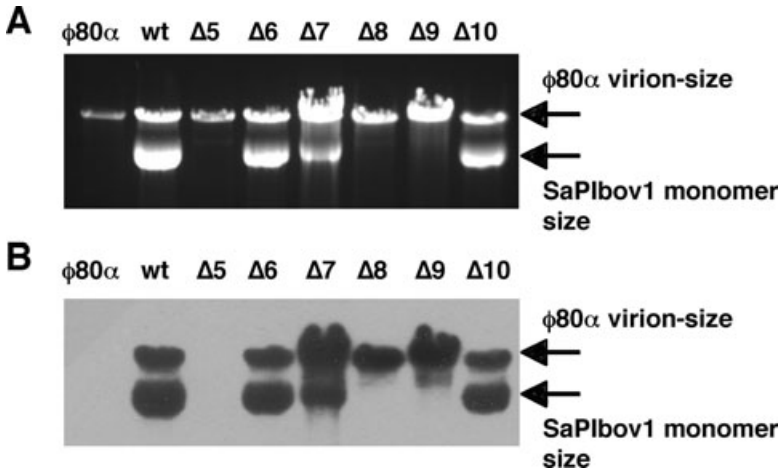

Fig. 6. Role of the different SaPIbov1-operon I ORFs in the production of SaPI-specific small capsids. DNA obtained from phage particles in the lysates of different lysogenic strains were analysed in agarose gels (A) or transferred for Southern blot studies using a probe specific for SaPIbov1 (B). Lane 1: RN27 (ϕ80α); lane 2: RN27 SaPIbov1; lane 3: RN27 SaPIbov1 ΔORF5; lane 4: RN27 SaPIbov1 ΔORF6; lane 5: RN27 SaPIbov1 ΔORF7; lane 6: RN27 SaPIbov1 ΔORF8; lane 7: RN27 SaPIbov1 ΔORF9; lane 8: RN27 SaPIbov1 ΔORF10.

required for SPST. In other words, it is likely that SaPIbov1 derivatives with mutations in either of these ORFs package their genome in full-sized phage capsids. This is supported by several lines of evidence, and is inferred (although it cannot be proven) by electron microscopy: a wt SaPIbov1 lysate consisted of about 80% small and 20% large particles (not shown), as has previously been seen with SaPI1 (Ruzin *et al*., 2001), whereas a lysate of SaPIbov1 ΔORF9 showed particles with a uniform size that appeared to have full-sized phage heads; however, measurements of such particles cannot be confidently compared with those obtained with a mixed lysate. Therefore, we extracted DNA from the particles produced by SaPIbov1 with the operon I mutations described above, in 80α lysogens, separated it on an agarose gel, transferred to nitrocellulose and hybridized with a SaPIbov1-specific probe. Although accurate measurements of DNA molecules of this size are not possible in a one-dimensional gel, it has been shown previously that phage genome sized DNA (~45 kb) is readily separable from the ~18 kb SaPI1-*tst::tetM* genome in a 0.7% agarose gel (Lindsay *et al*., 1998; Ruzin *et al*., 2001), and, moreover, that SaPI DNA is generally much more abundant than phage DNA in particles produced by 80α induction of SaPI1. As shown in Fig. 6, phage genome sized and SaPIbov1 genome sized DNAs were well separated and the following results were obtained: DNA of the size of 80α monomers was seen with all of the strains. Most significantly, the DNA in this position hybridized with a SaPIbov1 probe in all of the samples except that with 80α alone and that from the *Δter* mutant, confirming that with this mutant, SaPIbov1 DNA was not encapsidated to a detectable extent. A presumptively SaPIbov1 monomer-sized band was seen with the wt and with the ORF6, 7 and 10 mutants, consistent with the SaPIbov1 monomer band seen previously in cellular lysates prepared from MC-induced cells (Fig. 3). Remarkably, no SaPIbov1 monomer band was seen with the ORF8 and 9 deletions, confirming that these have defects in the formation of small SaPI capsids and that they encapsidate SaPIbov1 DNA in full-sized phage heads – presumably as SaPIbov1 trimers. In a similar analysis of phage particles produced with ϕ11-infected cells, the phage particle DNA from the ΔORF8 mutant, but not from the ΔORF9, contained SaPIbov1 monomers as well as ϕ11-sized molecules, suggesting that ΔORF8 has an incomplete defect in the ability to generate the small capside, whereas the ΔORF9 defect seems complete. This suggests that these two mutants are blocked at different steps in the formation of small SaPIbov1 capsids, and they are henceforth designated *cp2* and *cp1*, respectively, following their transcription order in the operon. It is also remarkable that a strong SaPIbov1 signal was seen in the band corresponding to 80α monomers with the wt and with the deletions in ORFs 6, 7 and 10, indicating that these strains package their DNA in full-sized as well as in SaPI-sized capsids.

Deletions in ORF6 and 10 did not demonstrably influence the SaPIbov1 ERP cycle, as their mutational inactivation had no effect on replication, encapsidation or SPST (data not shown) and their blotting patterns were the same as that of the wt island – so that their role in the SaPIbov1 ERP cycle remains to be determined. The ORF7 deletion (lane 5), however, had a much stronger signal at the phage monomer position than at the SaPI monomer position, suggesting that this mutant had a (partial) defect in small capsid assembly.

Cross-communication between SaPIbov1 and SaPI1

SaPI1 possesses an operon I homologue that is organized similarly to that of SaPIbov1, and we initiated a test for cross-complementation within operon I between these two SaPIs by testing SaPI1 for its ability to provide the SaPIbov1 functions eliminated by the operon I mutations described above. To perform this test, we transferred each of the SaPIbov1 operon mutants to strains JP1743 and

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JP1744, which contain an unmarked copy of SaPI1, and are lysogenic for 80α and ϕ11 respectively. These double-SaPI strains were then MC-induced and analysed for SPST and for the production of a SaPI band during phage growth by agarose gel electrophoresis. Each of the 80α lysogens showed a SaPI band that hybridized with a SaPIbov1-specific probe (data not shown) and showed high frequency SPST with TcR selection (not shown). This suggests that SaPI1, when induced by 80α, can provide the encapsidation functions lacking in the mutants defective in ORFs 5, 8 and 9, and cannot interfere with the normal encapsidation seen with the mutants defective in ORFs 6, 7 and 10. The observed cross-communication represent *trans*-complementation, and not recombination, as demonstrated by PCR analysis, using specific SaPIbov1 and SaPI1 probes, of the obtained colonies (data not shown).

With ϕ11 lysogens, however, no restoration of SaPIbov1 function was observed (not shown). Because ϕ11 cannot mobilize SaPI1, this means that induction is necessary for functional cross-communication.

What could be the selective advantage of control by LexA of SaPI packaging?

Indeed, why does LexA not control the genes involved in SaPI-replication? We suggest that upregulation of operon I by induction of the SOS response could ensure that SaPI DNA gets packaged, independently of replication, either in SaPI-specific small capsides or even in full-sized phage capsides, so long as it has been excised from its chromosomal site. We have shown elsewhere that SaPIbov2 can be transferred by phages that cannot induce its replication (Maiques *et al*., manuscript in preparation). Here, we show that an in-frame deletion affecting the SaPIbov1 replication initiator protein, Rep (ORF13, which has helicase activity; Úbeda *et al.* manuscript in preparation), eliminates SaPIbov1 replication (data not shown) but affects SPST only modestly (Table 1). Such results indicate that excision and encapsidation can occur in the absence of replication, which stands to reason from the biological standpoint, because transfer would seem, *a priori,* to be much more important than replication. As transfer is the only component of the SaPI ERP cycle that is SOS regulated, it is predicted that interference with SOS induction would block transfer in the absence as well as in the presence of replication. To test this prediction, we transferred the SaPIbov1*tst*::*tetM* ΔORF13 mutant to strain JP84 (LexA G94E, ϕ11 lysogen), MC-induced this strain and a congenic *lexA* wt strain and measured SPST frequencies of the resulting lysates. As shown in Table 1, blockage of LexA cleavage profoundly decreased SPST frequency, in the absence as well as in the presence of SaPI replication. As we have already encountered phages that can transfer SaPIs but cannot induce their replication, these results suggest that SOS induction of SPST is a key to the biological strategy of the SaPIs.

Discussion

In this report, we have shown that LexA represses SaPI operon I whose expression, alone among the SaPI transcripts, is therefore SOS-induced. Operon I contains six genes, at least four of which are involved in SaPI encapsidation and transfer. As one of these, ORF5, is highly similar to the terminase small subunit of phages from Gram-positive bacteria and is absolutely required for SaPI packaging and transfer, we suggest that it has the same function and designate it *ter.* Three others, ORFs 7, 8 and 9, are involved in the morphogenesis of characteristic small SaPI capsids and are therefore designated *cp3*, *cp2* and *cp1*, respectively, in the order of their transcription. Operon I is thus referred to as the encapsidation module. The mechanism by which small capsids are fashioned by these gene products from the same capsomeres as the full-sized phage heads (G. Christie, per. comm.) remains to be determined. Analogous to SaPI packaging, the satellite bacteriophage P4 requires all the morphogenic gene products provided by its helper phage P2 to assemble its own capsid, which is one-third the volume of P2 capsid (Shore *et al*., 1978). In contrast to the SaPIs, however, P4 only needs the product of one self-encoded gene, *sid*, to direct the assembly of the small capsids (Agarwal *et al*., 1990). Although the 5th and 1st genes of the operon, ORFs 6 and 10, have consensus translation start signals, it is not known whether they encode (functional) proteins. It is noted parenthetically that when SaPIs were first sequenced they were depicted with *int* at the right end and numbered from left to right (Lindsay *et al*., 1998). It was subsequently realized that all SaPIs are oriented so that their major transcription direction is parallel to that of chromosomal replication (Novick and Subedi, 2007), as are prophage genomes, which are conventionally depicted with *int* at the left end. We have subsequently reoriented the SaPI genomes according to this convention, so that for the early ones, including SaPI1, SaPIbov1 and SaPIbov2, the ORF numbers are in reverse order, with respect to the direction of transcription and chromosome replication. Because the original ORF numbers have been published, we do not propose to change them; however, when genetic designations are assigned, these are done with respect to the order of transcription. As ORF10 is at the 5′ end of operon I, the three ORFs that are involved in capsid formation are numbered in reverse order with respect to their ORF numbers. We also propose to designate orthologous genes in the different SaPI1s alphabetically. Thus, the operon I genes of SaPI

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will be *cp1A, cp2A, cp3A* and *terA* respectively; those of SaPIbov1 would use 'B', of SaPIbov2 'C', and those of SaPI2, 'D', etc.

Regarding the role of the SOS response in prophage and SaPI induction, there are two ways by which temperate phages may enter their vegetative replication cycle – either by SOS induction or following infection; similarly, the SaPIs enter their vegetative replication cycle either following SOS activation of a resident prophage capable of SaPI induction, or following infection by such a phage. In this report, we are concerned primarily with SOS induction. With SOS induction, cleavage of the prophage immunity repressor (cI) following activation of RecA is an essential first step in prophage induction, and is also required for SaPI induction. Ordinarily, the role of LexA in prophage induction is as a repressor of *recA.* Thus, the effect of non-cleavable LexA is to prevent any increase in RecA level following SOS induction, so that prophage induction would depend on cI cleavage by pre-existing RecA. In *S. aureus*, we have observed here that non-cleavable LexA decreases the yield of 80α pfu to a considerably greater extent than that of ϕ11 pfu, although the replication of both phages is affected, as is the replication of SaPIbov1 following induction either of ϕ11 or 80α. At the same time, non-cleavable LexA greatly reduces the yield of SaPIbov1 transducing particles either with 80α or ϕ11, to approximately the same extent and it reduces the frequency of ϕ69-induced transduction of several different SaPIs by 20- to 400-fold. Importantly, the only effect of non-cleavable LexA mutation on SaPI DNA replication is a reduction parallel to that of the reduction in phage DNA replication, suggesting that the only SaPI-specific effect of non-cleavable LexA is blockage of operon I expression. This was confirmed with a repressor-defective SaPIbov1 mutant that excises and replicates in the absence of any phage. With this mutant, in the absence of any phage, non-cleavable LexA had no effect on SaPIbov1 replication, even after MC induction, which it will be recalled, induces operon I expression in a $LexA^+$ background. These results together suggest that operon I induction by LexA cleavage is an important feature of SaPIbov1 encapsidation and is the only LexA-dependent SaPI function. We suggest that the biological basis for LexA regulation of SaPI encapsidation is that immediate upregulation of operon I by induction of the SOS response would provide an excess of SaPIbov1 Ter over the phage orthologue early in infection, thus favouring the encapsidation of SaPI DNA. In concordance with this idea, the lambda cI repressor is inactivated by RecA-dependent specific cleavage in a similar but far slower reaction than that observed for the inactivation of LexA (Kim and Little, 1993). Therefore, if this is also true for staphylococcal phage cI like repressors, the SaPIs would be able to express the genes needed for their encapsidation before even the phage is activated. It is interesting, in this connection, that operon I transcripts appear immediately following MC induction of SaPI1 in an 80α lysogen (P. Barry, Doctoral dissertation, NYUMS 2006), even though in a conventional phage transcription programme, the encapsidation module would be induced relatively late.

As phage infection does not involve any SOS function, it will be important to compare the SaPI packaging process following phage infection with that observed here following SOS induction. These experiments are currently in progress.

Finally, our studies and those of others suggest that LexA may have a wider role in bacterial gene regulation than has hitherto been imagined. For example, although the involvement of LexA in prophage induction is generally through its role as a repressor of *recA,* an interesting variation on this theme is SOS induction of the *V cholera* filamentous phage, ϕCTX. In this case, LexA is one of two repressors, both required for control of *rstA,* a gene required for phage induction. Cleavage of LexA following SOS induction is thus directly required for activation of this promoter, leading to the phage replicative cycle (Quinones *et al*., 2005). In contrast, we have shown in this study that LexA only regulates the SaPIs genes needed for their encapsidation but not for their replication. In our knowledge this is the first time that specific regulation by LexA of the encapsidation module of an MGE has been described. LexA has also been found to regulate genes that have nothing to do with the primary SOS response. Thus, it has recently been reported that LexA is a repressor of *S. aureus fnbB* (encoding fibronectin binding protein B) accounting for the observation that the fluoroquinone-induced SOS response enhances *S. aureus* binding to fibronectin (Bisognano *et al*., 2004).

Experimental procedures

Bacterial strains and growth conditions

Bacterial strains used in these studies are listed in Table S2 (*Supplementary material*). Bacteria were grown at 37°C overnight on TSA agar medium, supplemented with antibiotics as appropriate. Broth cultures were grown at 37°C in TSB broth with shaking (240 r.p.m.). Procedures for preparation and analysis of phage lysates, transduction and transformation in *S. aureus* were performed essentially as described (Novick, 1991; Lindsay *et al*., 1998; Ruzin *et al*., 2001).

Induction of prophages

Bacteria were grown in TSB broth to OD_{540} = 0.4 and induced by the addition of mitomycin C (2 μg ml^{-1}). Cultures were grown at 32°C with slow shaking (80 r.p.m.). Lysis usually

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occurred within 3 h. Samples were removed at various time points after phage induction, and standard SDS minilysates were prepared and separated on 0.7% agarose gels, as previously described (Lindsay *et al*., 1998).

DNA methods

General DNA manipulations were performed by standard procedures (Sambrook *et al*., 1989; Ausubel *et al*., 1990). Oligonucleotides tsst-3m/tsst-4c (Úbeda *et al*., 2003) and phi11-1m/phi11-2c (Table S3) were used to generate the specific SaPIbov1 and ϕ11 probes respectively. Labelling of the probes and DNA hybridization were performed according to the protocol supplied with the PCR DIG-DNA labelling and chemiluminescent detection kit (Roche).

Mobility shift assays

LexA-DNA complexes were detected by electrophoretical mobility shift assays (EMSA) using purified *B. subtilis* LexA protein, generously provided by Professor Roger Woodgate (Winterling *et al*., 1998), and PCR DIG-DNA labelled probes obtained using primers 7715-SaPIbov and 7883-SaPIbov for fragment A (FrgA), or primers 7883-SaPIbov and 8044-SaPIbov for fragment B (FrgB; Table S3), as previously described (Campoy *et al*., 2005).

Mutant promoters were obtained by overlap extension polymerase chain reaction (Higuchi *et al*., 1988), using the wild-type promoter as a probe and suitable oligonucleotides containing the Cheo box nucleotide substitution in their sequences. The obtained fragment was sequenced twice to further confirm the presence of the desired mutation.

Allelic exchange of SaPI genes

SaPI mutants were obtained in strain JP45 as previously described (Maiques *et al*., 2006). The oligonucleotides used to obtain the different mutants are listed in Table S3. Strain JP1847, encoding a non-cleavable LexA (LexA G94E), was previously obtained (Maiques *et al*., 2006).

Complementation of the mutants

The *ter* gene (ORF5) from SaPIbov1 was amplified with high-fidelity thermophilic DNA polymerase (Dynazyme Ext, Finnzymes) with the primers SaPIbov-121cK and SaPIbov-122mE (Table S3). The PCR product was cloned into the KpnI/EcoRI sites of pCN51 (Charpentier *et al*., 2004) and the resulting plasmid pJP368 was transformed by electroporation into *S. aureus* RN4220. Phage 80α was used to transduce pJP368 from RN4220 to the appropriate strains (Novick, 1991).

Real-time quantitative PCR

Procedures for preparation and analysis of mRNAs expression were essentially performed as previously described (Maiques *et al*., 2006). In each experiment, all the reactions were performed in triplicate. The relative transcriptional levels within distinct experiments were determined by using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). This method was also used in the analysis of the replication process of the different SaPIbov1 mutants. The results show the average $\pm$ SEM of at four independent experiments.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Analysis of the replication process of the different SaPIbov1 mutans.[a]

Table S2. Strains used in this study.

Table S3. Oligonucleotides used in this study.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2958.2007.05758.x
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