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Staphylococcus aureus Pathogenicity Island DNA Is Packaged in Particles Composed of Phage Proteins▽†

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Staphylococcus aureus **pathogenicity islands (SaPIs) have an intimate relationship with temperate staphylococcal phages. During phage growth, SaPIs are induced to replicate and are efficiently encapsidated into special small phage heads commensurate with their size. We have analyzed by amino acid sequencing and mass spectrometry the protein composition of the specific SaPI particles. This has enabled identification of major capsid and tail proteins and a putative portal protein. As expected, all these proteins were phage encoded. Additionally, these analyses suggested the existence of a protein required for the formation of functional phage but not SaPI particles. Mutational analysis demonstrated that the phage proteins identified were involved only in the formation and possibly the function of SaPI or phage particles, having no role in other SaPI or phage functions.**

The *Staphylococcus aureus* pathogenicity islands (SaPIs) are a large and coherent family of mobile phage-related pathogenicity islands that are found primarily in *S. aureus* and also in other gram-positive bacteria, including non-*S. aureus* staphylococci and lactococci (for a review, see reference 13). Most of them carry genes for one or more superantigens, and they are the primary cause of superantigen-induced diseases, especially toxic shock syndrome. SaPIs occupy specific chromosomal sites and are intimately related to certain temperate phages with which they share several essential functions: an integrase (absolutely required for excision and integration) (10, 14, 21); a replicon, including a specific replication origin, an initiator protein with helicase activity, and a primase (17); and a packaging module. The packaging module includes a terminase small subunit (absolutely required for encapsidation) and, in most but not all, morphogenetic genes that are responsible for the formation of small-headed phage-like particles into which their DNA is packaged (20). They also carry a pair of divergently transcribed regulatory genes that control their gene expression and appear to represent the primary regulatory interface with the inducing phage (18). An important feature of all SaPI genomes is the specific lack of a terminase large subunit and a portal protein coupled with the possession of a small terminase subunit, a combination that leads to efficient SaPI packaging at the expense of the phage. Consistent with this view is the prediction that SaPIs lack structural capsid proteins, so that their genomes are precisely designed for parasitization of the inducing phage, by means of which they are induced to excise and replicate and are encapsidated efficiently into phage-like particles, resulting in very high frequencies of intercell transfer. According to this understanding of the SaPI life cycle, it is strongly predicted that SaPI particles are composed of phage proteins and it is also likely that the SaPI genome contains functions that enable it to be packaged preferentially.

In this report, we confirm the above prediction for ϕ11 packaging of one of the SaPIs, SaPIbov1. We also demonstrate, using a mutational analysis, that the same proteins encoded by the distantly related phage 80α are used for SaPIbov1 and SaPI1 particle formation. This last result was anticipated by Tallent and coworkers, who analyzed by direct comparison of virion proteins the relationship between the compositions of SaPI1 transducing particles and those of helper phage 80α (16). However, in that previous study, no additional characterization of the proteins other than their identification was performed.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used in these studies are listed in Table 1. Bacteria were grown at 37°C overnight on Trypticase soy agar (Difco) supplemented with antibiotics for plasmid maintenance. Broth cultures were grown at 37°C in Trypticase soy broth with shaking (240 rpm). Procedures for preparation and analysis of phage lysates, transduction, and transformation in *S. aureus* were performed essentially as described previously (8, 12, 14).

Induction of prophages. Bacteria were grown in Trypticase soy broth to an optical density at 540 nm of 0.4 and induced by the addition of mitomycin C (MC) (2 mg/ml). Cultures were grown at 32°C with slow shaking (80 rpm). Lysis

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† Supplemental material for this article may be found at http://jb.asm.org/.

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TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Description	Reference
Strains		
RN4220	Restriction-defective derivative of RN450	6
RN451	RN450 lysogenic for ϕ11	11
RN10359	RN450 lysogenic for ϕ80α	17
JP1794	RN451(SaPIbov1 *tst*::*tetM*)	This work
JP2729	ϕ11 Δ31	This work
JP2731	ϕ11 Δ50	This work
JP2733	ϕ11 Δ34	This work
JP2735	ϕ11 Δ54	This work
JP2906	ϕ11 Δ45	This work
JP2930	ϕ11 Δ39	This work
JP3017	JP2906(SaPIbov1 *tst*::*tetM*)	This work
JP3018	JP2729(SaPIbov1 *tst*::*tetM*)	This work
JP3019	JP2731(SaPIbov1 *tst*::*tetM*)	This work
JP3020	JP2733(SaPIbov1 *tst*::*tetM*)	This work
JP3021	JP2930(SaPIbov1 *tst*::*tetM*)	This work
JP3022	JP2735(SaPIbov1 *tst*::*tetM*)	This work
JP3162	RN4220(pJP401)	This work
JP3163	RN4220(pJP416)	This work
JP3164	RN4220(pJP402)	This work
JP3165	RN4220(pJP403)	This work
JP3166	RN4220(pJP417)	This work
JP3167	RN4220(pJP404)	This work
JP3168	JP2906(pJP401)	This work
JP3169	JP2729(pJP416)	This work
JP3170	JP2731(pJP402)	This work
JP3171	JP2733(pJP403)	This work
JP3172	JP2930(pJP417)	This work
JP3173	JP2735(pJP404)	This work
JP3174	JP3168(SaPIbov1 *tst*::*tetM*)	This work
JP3175	JP3169(SaPIbov1 *tst*::*tetM*)	This work
JP3176	JP3170(SaPIbov1 *tst*::*tetM*)	This work
JP3177	JP3171(SaPIbov1 *tst*::*tetM*)	This work
JP3178	JP3172(SaPIbov1 *tst*::*tetM*)	This work
JP3179	JP3173(SaPIbov1 *tst*::*tetM*)	This work
JP3377	ϕ11 Δ *ter* small subunit	This work
JP3378	JP3377(SaPIbov1 *tst*::*tetM*)	This work
JP3565	ϕ80α Δ61	This work
JP3567	ϕ80α Δ68	This work
JP3569	ϕ80α Δ47	This work
JP3570	ϕ80α Δ62	This work
JP3576	ϕ80α Δ42	This work
JP3577	ϕ80α Δ53	This work
JP3578	JP3565(SaPIbov1 *tst*::*tetM*)	This work
JP3579	JP3576(SaPIbov1 *tst*::*tetM*)	This work
JP3580	JP3567(SaPIbov1 *tst*::*tetM*)	This work
JP3581	JP3569(SaPIbov1 *tst*::*tetM*)	This work
JP3582	JP3577(SaPIbov1 *tst*::*tetM*)	This work
JP3583	JP3570(SaPIbov1 *tst*::*tetM*)	This work
JP3584	JP3565(SaPI1 *tst*::*tetM*)	This work
JP3585	JP3576(SaPI1 *tst*::*tetM*)	This work
JP3586	JP3567(SaPI1 *tst*::*tetM*)	This work
JP3587	JP3569(SaPI1 *tst*::*tetM*)	This work
JP3588	JP3577(SaPI1 *tst*::*tetM*)	This work
JP3589	JP3570(SaPI1 *tst*::*tetM*)	This work
JP3602	RN10359(SaPI1 *tst*::*tetM*)	This work
JP3603	RN10359(SaPIbov1 *tst*::*tetM*)	This work
Plasmids		
pCN51	Expression vector for *S. aureus*	4
pJP401	pCN51-gene 45 ϕ11	This work
pJP416	pCN51-gene 31 ϕ11	This work
pJP402	pCN51-gene 50 ϕ11	This work
pJP403	pCN51-gene 34 ϕ11	This work
pJP417	pCN51-gene 39 ϕ11	This work
pJP404	pCN51-gene 54 ϕ11	This work

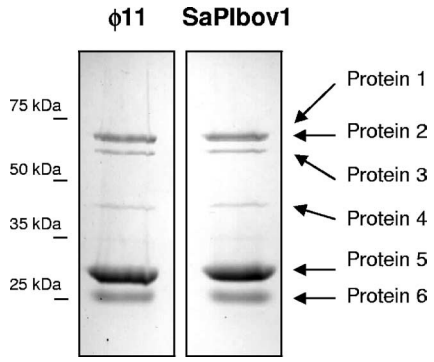

FIG. 1. Protein compositions of the phage- and SaPI-specific particles.

usually occurred within 3 h. Samples were removed at various time points after phage induction, and standard sodium dodecyl sulfate (SDS) minilysates were prepared and separated on 0.7% agarose gels as previously described (8).

DNA methods. General DNA manipulations were performed by standard procedures (3, 15). Oligonucleotides Orf12-2cB/Orf12-3mS and ϕ11-1m/ϕ11-2c (20) were used to generate the specific SaPIbov1 and ϕ11 probes, respectively. Labeling of the probes and DNA hybridization were performed according to the protocol supplied with the PCR-digoxigenin DNA-labeling and chemiluminescent detection kit (Roche).

Allelic exchange of phage genes. ϕ11 or 80α mutants were obtained in strains RN451 (ϕ11 lysogen) or RN10359 (80α lysogen) as previously described (9). The oligonucleotides used to obtain the different mutants are listed in Table S1 in the supplemental material. These oligonucleotides were designed using the published sequences for ϕ11 (accession number AF424781) or for 80α (accession number DQ517338).

Complementation of the mutants. ϕ11 genes were amplified with high-fidelity thermophilic DNA polymerase (Dynazyme Ext, Finnzymes) using the oligonucleotides listed in Table S1 in the supplemental material. PCR products were cloned into pCN51 under control of the P*cad* promoter (4), and the resulting plasmids (Table 1) were electroporated into *S. aureus* RN4220. These strains were used as recipients in the complementation studies. Phage ϕ11 was used to transduce the different plasmids from RN4220 to the appropriate donor strains (12).

Phage and SaPI purification and electron microscopy. The microscopy of SaPI and phage particles was performed as previously described (14). Particles were obtained from phage lysates by polyethylene glycol precipitation and CsCl step gradient centrifugation (15). Aliquots (10 μl) of fractions containing phage particles were applied on carbon-coated copper grids that were activated by glow discharge. After 30 s of incubation, the grids were briefly stained with a 2% water solution of phosphotungstic acid (Merck) (pH adjusted to 7.6 with NaOH), mounted on the microscope, and photographed.

In-gel enzymatic digestion and mass fingerprinting. Protein bands of interest were excised from a Coomassie blue-stained SDS-polyacrylamide gel and subjected to automated reduction, alkylation with iodoacetamide, and digestion with sequencing-grade bovine pancreatic trypsin (Roche) using a ProGest digestor (Genomic Solutions) according to the manufacturer's instructions. The tryptic peptide mixtures were dried in a SpeedVac and dissolved in 3.5 ml of 50% acetonitrile and 0.1% trifluoroacetic acid. An 0.85-ml portion of digest was spotted onto a matrix-assisted laser desorption ionization–time-of-flight sample holder, mixed with an equal volume of a saturated solution of a-cyano-4-hydroxycinnamic acid (Sigma) in 50% acetonitrile containing 0.1% trifluoroacetic acid, air dried, and analyzed with an Applied Biosystems Voyager-DE Pro matrix-assisted laser desorption ionization–time-of-flight mass spectrometer operated in delayed extraction and reflector modes. The peptide mass fingerprint obtained was compared with the known trypsin digest protein nonredundant databases (releases of February 2003) of SwissProt (http://us.expasy.org) or NCBI (http://www.ncbi.nlm.nih.gov) using the MS-Fit search engine of the Protein Prospector program (v.3.4.1) developed by the University of California at San Francisco and available at http://prospector.ucsf.edu. All searches were constrained to a mass tolerance of 50 ppm.

Collision-induced dissociation MS/MS. For structure assignment confirmation or peptide sequencing, the protein digest mixture was loaded in a nanospray capillary and subjected to electrospray ionization mass spectrometric analysis

TABLE 2. Protein composition of the SaPIbov1 particles

Protein	Nominal mass (kDa)	ϕ11		ϕ80α		Predicted function
		Protein	GenBank accession no.	Protein	GenBank accession no.	
1	73.6	pp45	AAL82273	pp61	ABF71632	Minor tail protein
2	66.8	pp54	BK006370	pp62	ABF71633	Minor tail protein
3	59.4	pp31	AAL82259	pp42	ABF71613	Phage portal protein
4	43.9	pp50	AAL82278	pp68	ABF71639	Tail fiber
5	36.7	pp34	AAL82262	pp47	ABF71618	Head protein
6	21.5	pp39	AAL82267	pp53	ABF71624	Major tail protein

using a QTrap mass spectrometer (Applied Biosystems) equipped with a nanospray source (Protana, Denmark). Doubly or triply charged ions selected after enhanced-resolution mass spectrometry (MS) analysis were fragmented using the enhanced product ion with Q0 trapping option. Enhanced resolution was performed at 250 amu/s across the entire mass range, a scanning mode that enables a mass accuracy of less than 20 ppm, making charge state identification reliable up to charge state 5. The term "enhanced product ion" refers to the performance of the PE-SCIEX-developed and patented LINAC (Q2) collision cell technology, which accelerates ions through the collision cell, thereby correcting the slow movement of ions due to high pressures existing within the chamber, and it provides high sensitivity and improved resolution in tandem MS (MS/MS) mode in comparison to triple quadrupoles without the LINAC collision cell. For MS/MS experiments, Q1 was operated at unit resolution, the Q1-to-Q2 collision energy was set to 35 eV, the Q3 entry barrier was 8 V, the linear ion trap Q3 fill time was 250 ms, and the scan rate in Q3 was 1000 amu/s. Collision-induced dissociation spectra were interpreted manually or using the on-line form of the MASCOT program (Matrix Science).

RESULTS

Construction and properties of a ϕ11 terminase small-subunit mutation. We have shown previously that the SaPI *ter* gene, encoding a homolog of the bacteriophage terminase small subunit, is absolutely required for SaPI packaging (20); only plaque-forming phage particles are produced upon induction of a *ter* mutant SaPI lysogen. It was therefore predicted that a mutation in the phage *ter* gene would result in a lysate composed exclusively of SaPI-containing particles. Accordingly, we constructed such a mutant using the pMAD method with a ϕ11 lysogen, RN451 (see Materials and Methods), introduced SaPIbov1-*tst*::*tetM* by transduction, and induced the prophage with MC. As predicted, the lysate contained <10 ϕ11 PFU/ml but contained ~10^8 SaPIbov1 transducing particles/ml. This lysate, concentrated by precipitation with polyethylene glycol and NaCl and purified by equilibrium sedimentation in CsCl (see Materials and Methods), was used as a source of SaPIbov1 particle proteins. Since we have shown previously that MC induction of a SaPIbov1-ϕ11 lysogen results in a lysate in which at least 90% of the particles produced are small-headed SaPIbov1 particles (19), we assume that lysates resulting from induction of the phage *ter* mutant will have at least this proportion of small-headed particles and therefore that a preparation of the proteins from such a preparation will be largely representative of these small-headed particles.

Identification of SaPIbov1 particle proteins. The SaPIbov1 transducing particles, purified from an MC-induced lysate of JP3378, a SaPIbov1-containing ϕ11 lysogen mutant with a mutation in the terminase small subunit of the phage, were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and compared with ϕ11 particle proteins obtained by induction of RN451, a ϕ11 wild-type lysogen. As shown in Fig. 1, the banding patterns obtained with the two preparations were identical, confirming the prediction that SaPI particles are composed of phage-encoded proteins and demonstrating that all of the detectable phage structural proteins are present in the SaPI particles.

We next extracted the five major SaPIbov1 protein bands and analyzed them by MS. Table 2 summarizes these results. As expected, each of these proteins corresponded to a phage protein, and the corresponding ϕ11 genes were readily identified from the published sequence (Fig. 2). The SaPIbov1 genome, however, contained no coding sequence corresponding to any of these proteins. Comparison of the phage capsid protein sequences with those predicted from the published staphylococcal phage genomes (7) indicated that these proteins are highly conserved among staphylococcal phages.

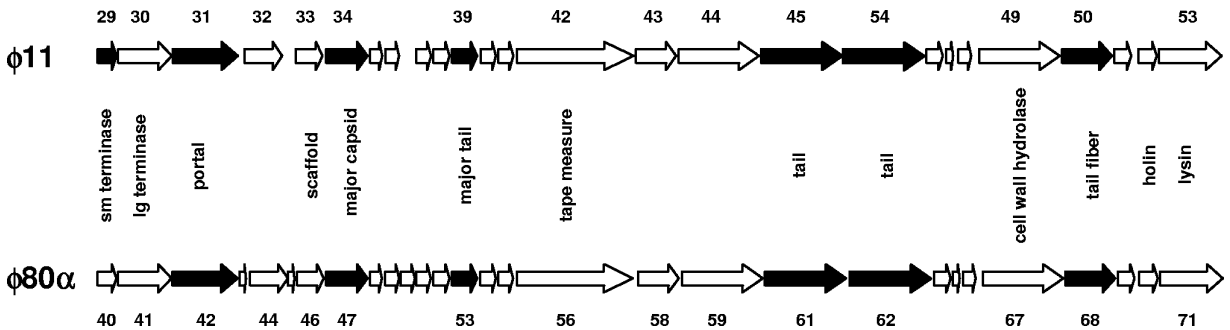

FIG. 2. Locations of genes encoding the proteins analyzed in this study. Arrows indicate predicted open reading frames, as annotated in the database entry (accession number AF424781 for ϕ11 and DQ517338 for 80α). Black arrows indicate genes deleted in this study. The open reading frame number for each gene is indicated.

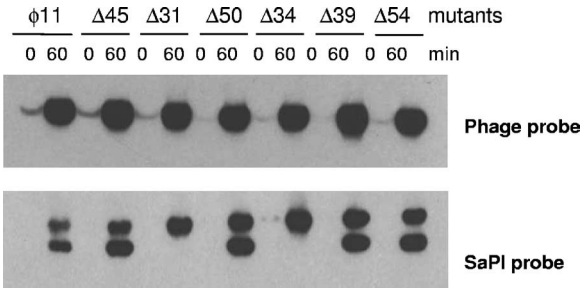

FIG. 3. Replication and encapsidation analysis of the different ϕ11 mutants. A Southern blot of the different ϕ11 mutant lysates carrying SaPIbov1 *tst*::*tetM*, obtained with samples taken 60 min after MC induction, separated on agarose, and blotted with a phage- or SaPIbov1-specific probe, is shown. The upper band is "bulk" DNA, including chromosomal, phage, and replicating SaPI; the lower band is SaPI linear monomers released from phage heads.

Proteins 1 and 2 were not sufficiently resolved by SDS-PAGE for MS on individual bands. Analysis of tryptic peptides from the mixture of proteins did allow their characterization. Protein 1 was identified as the predicted product of ϕ11_ORF45, as yet unidentified. Orf45 is located in a cluster containing genes predicted to encode components of the tail and base plate. We have no explanation for the failure of any of these to be present in the phage or SaPI particles. Protein 2 was not annotated in the original ϕ11 sequence (accession number AF424781) but has now been included in the GenBank database under the accession number BK006370, and we have included it in the analysis as pp54. This protein, present in other staphylococcal phages, is thought to be a minor tail protein. Protein 3, encoded by ϕ11_ORF31, is the putative portal protein, which not only connects head and tail but also is a component of the DNA encapsidation machinery. Protein 4, corresponding to the predicted product of ϕ11_ORF50, is a phage tail fiber protein. Protein 5, identified as the product of ϕ11_ORF34, is the major structural protein of the phage head. Protein 6 is predicted to be the product of ϕ11_ORF39, corresponding to the major tail protein.

Effects of phage mutants on SaPI transfer. To determine the roles of the different phage-encoded proteins in the SaPIbov1 excision-replication-packaging cycle, we generated an in-frame deletion in each of the genes in the RN451 ϕ11 prophage, using pMAD (Fig. 2). The resulting strains are JP2906 (gene 45 mutant), JP2735 (gene 54 mutant), JP2729 (gene 31 mutant), JP2731 (gene 50 mutant), JP2733 (gene 34 mutant), and JP2930 (gene 39 mutant) (Table 1). SaPIbov1 *tst*:*tetM* was then introduced into each mutant-containing strain, generating JP3017 to JP3022, respectively (Table 1).

The ϕ11 in-frame deletion mutants were each analyzed for two sequential and definable stages of phage and SaPI biology: replication and packaging. Each strain was MC induced, and screening lysates were prepared after 60 min, separated on agarose, stained, photographed, and then Southern blotted with a phage- or SaPIbov1-specific probe. We have not, in this presentation, specifically analyzed excision. We assume that mutants that produce a SaPI band or show significant replication must have been excised. Additionally, we assume that the mutants that produce a SaPI band are not affected in encapsidation, since the SaPI band is evidently produced by the disruption of intracellular SaPI heads (18). As shown in Fig. 3, none of the mutants was affected in phage or in SaPIbov1 DNA replication, although the gene 31 and 34 mutants failed to produce any SaPI band, suggesting encapsidation defects. Note, however, that in these mutants the phage and the SaPIbov1 DNAs were amplified to essentially the same degree as in the wild-type strain, JP1794 (ϕ11 SaPIbov1 *tst*::*tetM*). Although the gene 31 and gene 34 mutant cultures lysed at the usual time following MC induction, no phage particles could be detected in these lysates by electron microscopy, confirming their inability to produce capsids, as expected on the basis of their putative roles in phage morphogenesis (Table 2). Since all these strains lysed, it is concluded that the lysis functions of the phage proceed independently of capsid formation.

We next tested the mutants for the production of functional transducing/infective particles. As shown in Table 3, with the exception of the gene 50 mutant, which was unaffected, and the gene 39 mutant, which produced a few PFU, the rest of the mutants were unable to generate detectable plaque-forming phage.

With respect to SaPIbov1 transduction, the titer for the ϕ11 *ter* mutant was somewhat elevated and that for the gene 54 mutant was substantial but about 100-fold lower than that with the wild-type phage. No detectable transducing particles were produced by any of the other phage mutants. SaPIbov1 in the gene 39 mutant eliminated the few PFU produced by the mutant alone. Western blot analysis, using specific antibodies against pp50, of the lysate obtained from strain JP2731 (gene 50 mutant) confirmed the absence of the protein in the phage particles obtained from this strain (data not shown), suggesting

TABLE 3. Effect of phage mutations on ϕ11 titer and SaPIbov1 transfer

ϕ11	Donor strain	SaPIbov1	Titer[a]	
			Phage (PFU/ml induced culture)	Transduction (transductants/ml induced culture)
Wild type	RN451	−	1.1×10^8	
	JP1794	+	1.8×10^6	2.2×10^7
Δ*ter*	JP3377	−	<10	
	JP3378	+	<10	1.2×10^8
Δ45	JP2906	−	<10	
	JP3017	+	<10	<10
Δ54	JP2735	−	<10	
	JP3022	+	<10	1.2×10^5
Δ31	JP2729	−	<10	
	JP3018	+	<10	<10
Δ50	JP2731	−	7.6×10^7	
	JP3019	+	7.2×10^5	1.0×10^7
Δ34	JP2733	−	<10	
	JP3020	+	<10	<10
Δ39	JP2930	−	245	
	JP3021	+	<10	<10

[a] The means of results from three independent experiments are presented. Variation was within ±5% in all cases. RN4220 was used as the recipient.

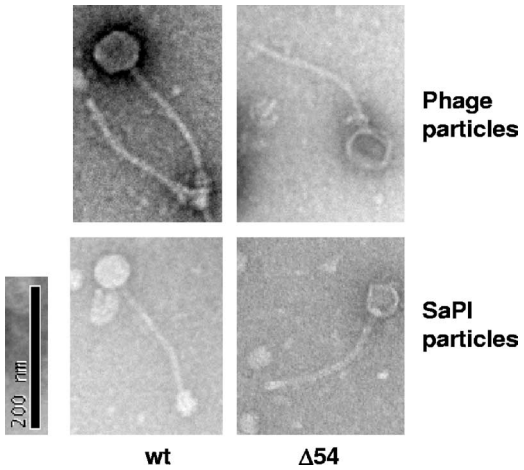

FIG. 4. Electron micrographs of ϕ11 gene 54 mutant lysates. Note the presence of SaPIbov1 particles (lower panels), which have smaller heads. wt, wild type.

that pp50 is not essential for the formation of functional phage particles, even though it is present in wild-type particles. Perhaps it is required for adsorption with certain host strains or affects the rate of adsorption.

The generation of SaPI transducing particles, but not phage particles, by the gene 54 mutant was strange and suggested that pp54 is necessary for the production of functional phage but not SaPI-specific particles; since it resembles a minor phage tail protein, it may be required for phage but not for SaPI particle adsorption. In view of this result, we analyzed by electron microscopy the phage and SaPI particles obtained from the wild-type ϕ11 and its derivative Δ54 mutant. As shown in Fig. 4, a structure at the end of the wild-type tail was absent from the mutant particles. However, this difference does not explain why the SaPI particles are functional and the phage particles are not.

Production of the characteristic SaPI band by phage with mutations in genes 39, 45, and 50 (Fig. 3) suggests that with these mutations, complete SaPI heads were produced and loaded with SaPI DNA but were transfer defective owing to mutations in these three tail genes, confirming that SaPI transfer involves the standard phage adsorption mechanism.

Complementation by cloned phage genes. To confirm that the observed effects of the different mutants on phage or SaPI transfer were specific for the mutated genes, we cloned the corresponding genes under the control of the cadmium resistance gene promoter (P*cad*) in plasmid pCN51 and transferred the resulting plasmids to strains containing the respective mutant prophages, generating stains JP3168 to JP3173. As shown in Table 4, each of the cloned genes enabled phage production by the corresponding mutant prophage, confirming that each mutation was fully responsible for its observed phenotype.

As part of the analysis of these phage mutants, we introduced SaPIbov1-*tst*::*tetM* into each of the strains containing the complementing plasmids and the mutant ϕ11 lysogens, generating JP3174 to JP3179, respectively, and tested them for the production of plaque-forming and SaPI transducing particles following MC induction. In all cases, as shown in Table 4, the phage titer was sharply reduced, as is ordinarily seen with a SaPI, and is illustrated by the combination of wild-type ϕ11 and SaPIbov1. With the exception of the gene 34 mutant, the SaPI transducing titers of the complemented mutant strains were restored essentially to normal levels. Moreover, the SaPI titers for most of the complemented mutants were usually 100- to 1,000-fold higher than the phage titers, suggesting that the well-known preferential packaging of SaPI is enhanced by even the modest shortages of capsid proteins seen with the complemented phage mutations. This effect is minimal with the gene 34 mutant, for which SaPIbov1 sharply reduces the phage titer

TABLE 4. Effect of complementation in ϕ11 and SaPI titer

Donor strain	ϕ11	Plasmid	SaPI	Titer[a]	
				Phage (PFU/ml induced culture)	Transduction (transductants/ml induced culture)
RN451	Wild type			1.1×10^8	
JP1794	Wild type		SaPIbov1	1.8×10^6	2.2×10^7
JP3168	Δ45	pCN51-gene 45		2.2×10^7	
JP3174	Δ45	pCN51-gene 45	SaPIbov1	6×10^4	7×10^6
JP3173	Δ54	pCN51-gene 54		2.6×10^7	
JP3179	Δ54	pCN51-gene 54	SaPIbov1	1×10^5	7×10^6
JP3169	Δ31	pCN51-gene 31		4.5×10^7	
JP3175	Δ31	pCN51-gene 31	SaPIbov1	1.8×10^5	5.2×10^7
JP3170	Δ50	pCN51-gene 50		3.5×10^7	
JP3176	Δ50	pCN51-gene 50	SaPIbov1	1.4×10^5	1.5×10^7
JP3171	Δ34	pCN51-gene 34		2.7×10^7	
JP3177	Δ34	pCN51-gene 34	SaPIbov1	3×10^3	1.6×10^4
JP3172	Δ39	pCN51-gene 39		2.2×10^7	
JP3178	Δ39	pCN51-gene 39	SaPIbov1	1×10^5	9×10^6

[a] The means of results from three independent experiments are presented. Variation was within ± 5% in all cases. Complemented RN4220 was used as the recipients.

TABLE 5. Effect of phage mutations on ϕ80α titer and SaPI transfer

ϕ80α	Donor strain	SaPI	Titer[a]	
			Phage (PFU/ml induced culture)	Transduction (transductants/ml induced culture)
Wild type	RN10359		1.2×10^{10}	
	JP3602	SaPI1	9.8×10^{8}	4.8×10^{7}
	JP3603	SaPIbov1	5.8×10^{9}	1.3×10^{7}
Δ61	JP3565		<10	
	JP3584	SaPI1	<10	<10
	JP3578	SaPIbov1	<10	<10
Δ62	JP3570		<10	
	JP3589	SaPI1	<10	1.4×10^{5}
	JP3583	SaPIbov1	<10	2.8×10^{4}
Δ42	JP3576		<10	
	JP3585	SaPI1	<10	<10
	JP3579	SaPIbov1	<10	<10
Δ68	JP3567		4.0×10^{10}	
	JP3586	SaPI1	5.3×10^{8}	1.9×10^{7}
	JP3580	SaPIbov1	3.0×10^{9}	4.3×10^{7}
Δ47	JP3569		<10	
	JP3587	SaPI1	<10	<10
	JP3581	SaPIbov1	<10	<10
Δ53	JP3577		<10	
	JP3588	SaPI1	<10	<10
	JP3582	SaPIbov1	<10	<10

[a] The means of results from three independent experiments are presented. Variation was within ±5% in all cases. RN4220 was used as the recipient.

but is not preferentially packaged even to the extent seen with the wild-type phage.

Effects of phage 80α mutants on SaPI transfer. In a recent study with phage 80α and SaPI1, Tallent and coworkers identified 12 virion proteins from a sample containing SaPI1 particles (16). Since the most abundant proteins reported were homologous to those identified in this study and since no additional characterization of these proteins was performed in the previous study, we decided to obtain mutants with mutations in the 80α genes showing identity with the ϕ11 genes characterized here. The relationship between the two phages is shown in Table 2 and Fig. 2. For that, we generated an in-frame deletion in each of the genes in the RN10359 80α prophage, using pMAD (2). The resulting strains are JP3565 (gene 61 mutant), JP3576 (gene 42 mutant), JP3567 (gene 68 mutant), JP3569 (gene 47 mutant), JP3577 (gene 53 mutant), and JP3570 (gene 62 mutant) (Table 1). Since phage 80α induces the excision-replication-packaging cycle both of SaPI1 and SaPIbov1, *tst*:*tetM* derivatives of both islands were then introduced into each mutant-containing strain, generating JP3578 to JP3589, respectively (Table 1).

We next tested the mutants for the production of functional transducing/infective particles. As shown in Table 5, and as previously reported for the ϕ11 gene 50 mutant, the 80α gene 68 mutant was unaffected. Regarding the rest of phage mutants, no detectable phage or SaPI transducing particles were produced by any of the other phage mutants, except for the 80α gene 62 mutant. As described for the ϕ11 gene 54 mutant, 80α gene 62 is necessary for the production of functional phage but not SaPI-specific particles.

DISCUSSION

In this report, we have confirmed with the combination of SaPIbov1 and ϕ11 the strong prediction that SaPI particles are composed entirely of phage proteins, of which six were identified by SDS-PAGE analysis of purified SaPI particles. These six include the major head and tail proteins, the portal protein, a tail fiber protein, and two minor tail proteins. Essentially the same proteins, encoded by a distantly related phage, 80α, comprise the particles of the distantly related SaPI1 (16). These proteins appear to represent all of the proteins comprising the phage capsid in both cases. The heads of the two types of particles thus appear to be comprised of a single protein, the ϕ11 gene 34 product. Most of the known SaPIs contain three highly conserved genes, *cp1*, *cp2*, and *cp3*, that are required for the assembly of pp34 into small capsids (20); pp34 assembly into the standard phage capsids presumably involves a phage-encoded size-determining scaffold. It is not known whether pp34 can be assembled into more than two differently sized capsids; it is notable, however, that SaPIbov2, which has a 27-kb genome, lacks the capsid assembly determinants and is efficiently packaged into full-sized phage capsids (10). The staphylococcal phage-SaPI system provides an interesting contrast with the *Escherichia coli* P2/P4 phage system. In both cases, the parasitic element, SaPI or P4, encodes proteins that

remodel the phage capsid to accommodate the smaller genome of the parasite but are not contained in the mature particles. The remodeling protein, Sid, of P4 forms an external scaffold for small capsid assembly (1). As noted, the SaPIs encode three proteins that are required for capsid morphogenesis; however, the mechanism in this case has yet to be identified.

Several of the predicted tail proteins had rather unusual properties. The tails of both types of particles appear identical in the electron microscope and are presumably comprised of the same proteins; however, one of these, pp54, annotated in other phage genomes as a minor tail protein and required for the formation of functional phage particles, is not absolutely required for the formation of functional SaPI particles, though the SaPI transducing titer is reduced about 100-fold by the gene 54 mutation. Since one would assume that the adsorption-DNA insertion process would be identical for the two types of particles, this effect is not readily explained.

Surprisingly, a mutation affecting ϕ11 pp50 or ϕ80α pp68, a protein that is similar to tail fiber proteins of other phages, had very little, if any, effect on the production of functional phage or SaPI particles. Tail fiber proteins are responsible for the recognition of the host receptor in some phages, such as phage λ or T5 (5, 22). However, our data suggest that these proteins are not essential for the infectivity of ϕ11 or ϕ80α, at least for the indicator strain used in this study. Since the specificity of staphylococcal phage adsorption is poorly defined, it is possible that this protein may be required with other strains.

The biological significance of small SaPI-specific capsids is not entirely obvious, since several SaPIs do not produce them and are encapsidated in full-sized phage particles with equal efficiency (10), as are SaPIs with mutations in the morphogenesis determinants (20). One possible advantage of the small capsids is that they provide a competitive advantage for the SaPI over the inducing phage; even though phage DNA can be encapsidated in the small particles, only about one-third of the phage genome can be accommodated, and so a rather high multiplicity of these would be required to produce a functional phage genome.

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