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The phage-related chromosomal islands of Gram-positive bacteria

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Abstract

The phage-related chromosomal islands (PRCIs) were first identified in *Staphylococcus aureus* as highly mobile, superantigen-encoding genetic elements known as the *S. aureus* pathogenicity islands (SaPIs). These elements are characterized by a specific set of phage-related functions that enable them to use the phage reproduction cycle for their own transduction and inhibit phage reproduction in the process. SaPIs produce many phage-like infectious particles; their streptococcal counterparts have a role in gene regulation but may not be infectious. These elements therefore represent phage satellites or parasites, not defective phages. In this Review, we discuss the shared genetic content of PRCIs, their life cycle and their ability to be transferred across large phylogenetic distances.

Horizontal gene transfer has an extremely important role in bacterial evolution. It has been estimated that some 20% of the extant genetic content of any given bacterial species has been acquired from other organisms[1]. Perhaps half of this 20% consists of mobile genetic elements (MGEs) that have moved freely within and between species and have occasionally crossed boundaries between genera.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Genome: http://www.ncbi.nlm.nih.gov/sites/entrez?db=genome
phage 80α | phage 85 | phage ϕ13 | phage P4 | phage PH15
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/genomeprj
Bacillus subtilis | Enterococcus faecalis | Escherichia coli | Lactococcus lactis | Listeria monocytogenes | Mycobacterium tuberculosis | Staphylococcus aureus | Staphylococcus chromogenes | Staphylococcus epidermidis | Staphylococcus xylosus | Streptococcus pyogenes | Sulfolobus islandicus
GenBank: http://www.ncbi.nlm.nih.gov/genbank
NC_007622

FURTHER INFORMATION
Richard P. Novick's homepage: http://saturn.med.nyu.edu/research/mp

SUPPLEMENTARY INFORMATION
See online article: S1 (figure) | S2 (figure) | S3 (table) |S4 (figure) | S5 (table) | S6 (table) | S7 (table)
ALL LINKS ARE ACTIVE IN THE ONLINE PDF

In facultative pathogens, MGEs are largely responsible for antibiotic resistance, environmental adaptations and the wide range of adaptations to life in host tissues that we perceive as pathogenesis. Among these pathogens is Staphylococcus aureus, which is a major scourge of the hospital environment and has become increasingly important of late as a cause of infections among otherwise healthy individuals. Not only are some strains resistant to methicillin and oxacillin owing to the acquisition of the staphylococcal cassette chromosome *mec* (SCC*mec*) element[2], but also the species as a whole has increased its virulence and transmissibility. A possible contributor to this increased virulence is Panton-Valentine leukocidin (PVL), the prophage-encoded toxin responsible for necrotizing pneumonitis, which can be fatal within 24–48 hours, as recently described[3]. Superantigens are among the other important staphylococcal virulence factors; they are responsible for food poisoning, toxic shock syndrome (TSS) and necrotizing fasciitis. Toxic shock syndrome toxin (TSST1) is particularly insidious, especially in surgical wounds, because it inhibits the production of most other virulence factors and, consequently, causes little inflammation[4].

All known classes of MGEs in *S. aureus* and other pathogenic bacteria, including temperate phages, plasmids, transposons and other horizontally acquired units, may contribute to pathogenesis; it is particularly striking that nearly all of the bacterial toxins that cause specific toxin-mediated diseases, such as PVL pneumonia, TSS, necrotizing fasciitis and food poisoning — known as toxinoses — are encoded by MGEs[5]. Although there are well-defined classes of MGEs, there are also transitional forms that can be classified as chromosomal islands; these are discrete chromosomal segments that have been acquired by horizontal transfer, that are usually flanked by direct repeats and that lack essential genes. Chromosomal islands range in size from two genes (called ‘islets’) to several hundred genes. Most do not seem to be mobile anymore, probably owing to the loss of mobilization functions. Those chromosomal islands that are mobile use either phage-mediated transfer or self-coded conjugative transfer machinery to move between cells (DNA-mediated transformation is not known to be an MGE-specific mode of transfer). The discovery of the highly mobile *S. aureus* pathogenicity islands (SaPIs), which carry genes encoding TSST1, staphylococcal enterotoxin B (SEB; also known as EntB) and other superantigens[6], was an important advance in our understanding of the mobile character of pathogenicity islands (BOX 1). These elements do not encode any machinery for their horizontal transfer and, instead, hijack the capsids of phages (known as helper phages) for their transduction. SaPI gene expression is carefully regulated to take advantage of the lytic cycle of the helper phage and to maximize the transfer of progeny. Many *S. aureus* genomes contain one or more SaPIs, including several that do not carry any genes encoding superantigens or any other discernable accessory genes. Virtually all clinical toxic shock-causing isolates contain two or more SaPIs with various combinations of genes, suggesting that there has been extensive recombination between SaPIs[7].

The recent identification of closely related elements in other staphylococcal species[8,9] and in other genera[10] (BOX 2) has prompted us to suggest a general designation for all these elements of ‘phage-related chromosomal islands’ (PrCIs), owing to the similarity of these elements to phages (BOX 1). In Staphylococcus epidermidis the PrCI SePI-I[11] encodes the toxins staphylococcal enterotoxin C3 (SEC3; also known as EntC) and staphylococcal enterotoxin L (SEL); this is the first example of super antigen production by staphylococci other than *S. aureus*. SePI-I was probably acquired from *S. aureus*, but it lacks many of the genes that are found in SaPIs and is probably non-transmissible. PCrIs have also been identified in recently sequenced isolates of Lactococcus lactis (r.P.N., unpublished observations), *Streptococcus* spp.[10] and Enterococcus faecalis (J.r.P., unpublished observations).

All of the sequenced staphylococcal genomes contain two other pathogenicity islands, νSaα and νSaβ, which contain sets of serine protease-like and superantigen-like genes, respectively, but lack flanking direct repeats and mobilization functions[12], and it is unclear whether these elements are acquired through horizontal transfer.

In this review, we address the basic molecular genetics of SaPIs, which are prototypical PrCIs, focusing on their genomic organization and regulation, their mobility (including the potential to disseminate superantigens and other virulence factors), and their interaction with and use of helper phages. we also comment briefly on the trans-genera transfer of SaPIs and on the existence of similar elements in other bacteria.

Genome organization

Conserved core genes

All SaPIs share a core set of genes that regulate their life cycle (FIG. 1; TABLE 1). At the left end of the SaPI genome is a homologue of the typical phage integrase gene (*int*), which is required for the integration of the element. Adjacent to *int*, sometimes with one or more intervening accessory genes, are two divergently oriented promoters that regulate two major transcription units: *stl* on the left, and *str* on the right. These two genes resemble the divergent *cI* and *cro* genes, respectively, of temperate coliphages, which encode regulator proteins. Stl is a master repressor for the SaPI excision–replication–encapsidation cycle[13] and is de-activated during phage induction; the main function of Stl is to repress transcription of *str*. To the right of *str* is *xis* (which encodes excisionase), followed by the replication module, consisting of *pri*, which encodes a primase, *rep*, which encodes a replication initiator, and a SaPI-specific origin. In some cases the primase is fused to the replication initiator; it enhances replication but is not absolutely required[13]. rep is similar to replicon-specific replication initiators of other types of replicons and has a helicase activity[13] that is similar to that of α-protein of phage P4 (REF. 14). The replication origin consists of two sets of inversely oriented hexanucleotide-to-octanucleotide `iterons` flanking an AT-rich region (see Supplementary information S1 (figure)). In SaPIbov1, gene 12, called *pif*, is to the right of the replication origin and is responsible for interference with phage growth[15]. This gene is followed by gene 11, which encodes a protein of unknown function and an operon that is involved in genome packaging. The operon is controlled by the `SOS response`-specific repressor LexA[13,15–17] and includes *terS*, a close homologue of the terminase small subunit of several phages from Gram-positive bacteria. Interestingly, most of the predicted SaPI-specific genes encoding proteins with unknown functions do not have close orthologues. These gene identifications discussed above are based mostly on experiments performed with SaPIbov1, but many have been confirmed in other SaPIs, primarily SaPI1 (REF. 7), SaPI2 (REF. 7) and SaPIn1 (J.r.P., unpublished observations).

PRCI-specific accessory genes

The accessory genes can be located at either or both ends of the PrCI. The putative functions of many of these genes have not been analysed and are assigned on the basis of homology, location or both. In addition to superantigen genes, PrCIs carry genes for antibiotic and phage resistance as well as genes encoding biofilm-inducing proteins, a putative ferrichrome ATP-binding cassette (ABC) transporter, a membrane protein (Mmp) and a putative acetyl transporter (Act) (TABLE 2), although several PrCIs lack any identifiable accessory genes. orthologues of these genes can be found in other genomes, suggesting that some random genetic exchange may be responsible for their acquisition by PrCIs, although several of the most important staphylococcal superantigen genes, including *tst* (encoding TSST1), *seb* and the *sec* genes, are found only in SaPI genomes.

PRCIs of other Gram-positive bacteria

Several putative PrCIs have been identified in other Gram-positive bacterial genera, either by searching the published genome sequences or by tracking phenotypes. These share the basic features of the staphylococcal pathogenicity islands but differ in certain details (FIG. 1b). The two PCrIs identified in *L. lactis* most closely resemble the SaPIs; the other non-staphylococcal PrCIs usually lack an identifiable *terS* homologue. It is possible that this function is carried out by a protein that does not resemble TerS or that these PrCIs use alternative packaging strategies. A genetic element in Streptococcus pyogenes, SF370.4, which was described by McShan and colleagues as a prophage remnant[10], has all of the genomic features of a PrCI. we suggest, with the authors' concurrence (w. McShan, personal communication), that it be re-designated as PrCI SpyCIM1. unlike most PrCIs, however, SpyCIM1 excises and reintegrates during growth[10]. *E. faecalis* carries the PrCI EfCI, which can be transferred to and replicate in *S. aureus* (J.r.P., unpublished observations).

The SaPI lifestyle

SaPIs reside stably in the host chromosome, like prophages, but their excision and replication are not induced by the SoS response; instead, they require a helper phage. Following superinfection by a helper phage or induction of a resident helper prophage by the SoS response, the SaPI genome is excised by Int and Xis, presumably using the `Campbell mechanism`[19], and replicates independently to form a concatamer. Alongside the production of phage virions, smaller SaPI capsids are produced from phage proteins. Packaging of SaPI DNA into these phage capsids is initiated by cleavage of the DNA at SaPI *pac* sites (terminase recognition sites) by TerS. Phage-induced lysis releases both mature phages and SaPI particles (FIG. 2a). on entry into a new host cell, SaPI DNA follows a replicative pathway if the DNA is accompanied by an incoming phage genome and an integrative pathway if not (FIG. 2b,c).

Integration

Chromosomally located MGEs — such as PrCIs, SCC*mec*s, ICEs (integrative and conjugative elements) and prophages — that encode and use sites-pecific integrases integrate at a site (known as the *attC* site) that is determined by the specificity of the integrase, and these elements are flanked by directly repeated sequences that comprise the core integrase recognition site. SaPIs require only integrase for insertion, although integration seems to be inefficient, as incoming SaPI DNA persists for several hours in the autonomous (but non-replicating) state[20], perhaps because integrase is expressed poorly under these conditions.

The known SaPIs occupy six different *attC* sites in the staphylococcal genome[21] (TABLE 3; see Supplementary information S2 (figure)); these sites correspond to the *attB* sites for bacteriophage integration. The SaPIs each contain a corresponding insertion site sequence, *attS*, which corresponds to the classical *attP* integration site of bacteriophages. The six different *attC* sequences are unrelated to each other and occur only once in each of the sequenced *S. aureus* genomes. when *S. aureus* carries more than one SaPI, as is common among the sequenced strains (TABLE 3), the SaPIs occupy different chromosomal *attC* sites; no strain has been found to contain two SaPIs at the same *attC* site to date.

When the SaPI1 *attC* site is deleted[22], SaPI1 inserts into secondary *att* sites at nearly the same collective frequency as it inserts into its primary site[22] (see Supplementary information S3 (table)). The sequence conservation at the core of the secondary *att* sites is low, and the sequences flanking the core are not at all conserved. The specificity of the SaPI1 Int

therefore seems to be between that of a classical integrase, which has a very high specificity, and a transposase, which has a much lower specificity.

Regulation

After integration of the SaPI into the host chromosome, SaPI gene expression is repressed by the master regulator Stl (FIG. 1,2a). Mutational inactivation of the Stl of SaPIbov1 (REF. 13) and SaPI1 (A. Matthews and r.P.N., unpublished observations) leads to SaPI excision and replication in the absence of a helper phage. This is probably due to reversible integration–excision, because the autonomous (that is, unintegrated) form is extremely unstable in the absence of a functional Int (see below). Therefore, the primary, if not the only, regulatory function of the helper phage is to relieve Stl-mediated repression. However, phages differ in their ability to induce SaPIs; in one study, phage 80α could induce all of the five SaPIs tested, whereas phage ϕ11 induced only SaPIbov1 (REF. 23) and phage 85 could not induce any.

The site of action of Stl is in the intergenic region between the *stl* and *str* promoters, suggesting that it regulates transcription from both promoters[23]. In the integrated state, none of the rightward genes are expressed, whereas *int*, which always lies to the left, is expressed constitutively but at a low level from the *stl* promoter (r. Adhikari and r.P.N., unpublished observations). Although *str* is negatively regulated by Stl[24], the regulatory function of Str is unclear, as an *str* mutation in SaPIbov1 has no obvious effect on the SaPI replication cycle[13].

Relief of repression by helper phages

Phage 80α mutants that were selected for their ability to form plaques on SaPIbov1-containing staphylococcal strains were all found to contain mutations in *dut*, the phage gene encoding duTPase. These mutations eliminated SaPIbov1 induction. Furthermore, the addition of duTPases from either phage ϕ11 or phage 80α enabled non-helper phages 147 and 85 to induce SaPIbov1 (REF. 24). Interestingly, duTPase is a bifunctional 'moon-lighting' protein, as mutations affecting duTPase activity but not SaPIbov1 induction, and vice-versa, have been isolated[24]. Although duTPase is highly conserved among staphylococcal and other phages, there is a sharply divergent central region of about 40 amino acids (see Supplementary information S4 (figure)) that is involved in SaPI mobilization. This region is absent from non-inducing phages, such as the *S. epidermidis*-infecting phage PH15, and from chromosomally encoded duTPases, such as that of Mycobacterium tuberculosis[25]. duTPase relieves SaPIbov1 repression by binding to Stl, thus activating transcription from both the *stl* and *str* promoters[24]. Remarkably, phage 80α Δ*dut* does not induce SaPI2 but does induce SaPI1 and SaPIbov2 (see Supplementary information S5 (table)), suggesting that different phage functions are required for the induction of different SaPIs. These were identified by selection for phage 80α mutants that were able to form plaques on staphylococcal strains containing the other SaPIs[24]. The gene responsible for SaPI1 induction is *sri*, which encodes a DnaI-binding protein that blocks bacterial DNA replication, and the gene responsible for SaPIbov2 induction is *orf15*, which encodes a small protein of unknown function. Both of these genes are absent from the phage ϕ11 genome but present in the genomes of many other staphylococcal phages. Phage 80α with mutations in *sri* are defective in the induction of SaPI1 but not of SaPIbov1 or SaPI2; mutations in *orf15* eliminate SaPIbov2 induction but have no effect on the other pathogenicity islands (see Supplementary information S5 (table)). In each case, expression of the cloned gene induces excision and replication of the responsive SaPI[24]. It will be interesting to unravel the evolutionary process underlying the development of the SaPI–phage interactions, including the induction of SaPIs by SaPI-specific phage proteins and the interference of specific SaPI-encoded proteins with phage reproduction.

Excision

Excision requires an Xis function[13,26]. As *xis* is repressed by Stl, the general SaPI repressor, excision occurs only following induction; excision of the uninduced SaPI genome is very rare or undetectable. This serves the needs of the island: spontaneous excision in the presence of a functional repressor would generally cause loss of the island if cell division were to occur before it could reintegrate. Indeed, at least two of the known SaPIs undergo rare spontaneous excision and are slightly unstable, whereas others do not and are very stable[20,26]. Constitutive expression of *int* also serves the needs of the island, as it must integrate following cell entry or it will be lost.

Replication

After excision, SaPIs are replicated extensively, ensuring that many particles will be released from the cell. SaPI replication is similar to that of other small bacterial replicons. SaPIs encode replicon-specific rep proteins with matched replication origins (*ori* sites). origin specificity is determined by the sequence and arrangement of iterons in the *ori* (see Supplementary information S1 (figure)) and a short carboxy-terminal region of rep[27]. Initiation requires the AT-rich region between the iterons and the inversely oriented iterons themselves. we propose that rep binds to the iterons, inducing melting of the AT-rich region and initiating replication through the helicase activity of the protein. A host DNA polymerase then replicates the DNA, leading to concatemer formation by an unidentified mechanism. During replication several hundred copies of SaPI DNA are produced. In the absence of a replicating phage, the incoming SaPI DNA circularizes before integration[20]. This circularization presumably occurs through recombination between the redundant termini, and it must involve an unknown recombinase, as SaPI transfer is recombinase A (recA) independent[6].

The *pri–rep–ori* region of SaPIbov1 can promote the replication of an Escherichia coli vector in *S. aureus*[27]. However, the plasmid is extremely unstable, with a segregation probability of about 0.4; this is probably because the replication product, like that of the intact SaPI, is a linear concatemer and so is likely to have a very low copy number and to be unable to segregate normally to daughter cells.

Initiation of the SaPI replication cycle can be induced in three ways: following SoS response-mediated induction of a helper prophage present in the same cell as the SaPI (FIG. 2a); following superinfection of a SaPI-containing non-lysogen by a helper phage; or following the joint entry of SaPI and helper phage DNAs into a cell lacking both[5] (FIG. 2c). SpyCIM1 is an example of a SaPI that is induced after activation of the SoS system, but the possible participation of endogenous helper prophages has not yet been addressed. After insertion into the chromosome, SpyCIM1 interrupts the DNA mismatch repair (*mutS–mutL*) operon, blocking expression of *mutL*[10]. It can excise spontaneously during exponential growth, thereby transiently activating *mutL* expression. It then replicates transiently and is reintegrated, shutting off *mutL*, as soon as the bacterium exits the exponential phase.

when the SaPI replication cycle is initiated by SoS response-mediated induction of a helper phage or by superinfection with a helper phage, the initial event is relief of SaPI repression, but in the case of joint entry of the SaPI and the helper phage, repression is not established. In the absence of a helper phage, incoming SaPI1 DNA does not detectably replicate, almost certainly because *stl*-mediated repression is rapidly established. In all three scenarios, linear monomeric SaPI DNA is produced; it appears immediately following infection but much later following induction of an endogenous SaPI, when it results from the disruption of intracellular SaPI particles[16]. After SoS response-mediated induction of a helper phage or superinfection by a helper phage, it is assumed that phage-induced SaPI excision occurs by

the Campbell mechanism, but the expected circular excision product is not detected. Instead, replicating SaPI DNA migrates with the bulk DNA in agarose gels, suggesting that the SaPI is present as concatenated, linear copies. This indicates that SaPI replication may be initiated on either linear or circular DNA. Following joint entry of a SaPI and a helper phage, the amount of linear DNA increases, possibly reflecting SaPI replication. Late in infection (at 50–60 minutes after infection), supercoiled SaPI monomers appear and SaPI DNA can be detected in the bulk DNA, indicating that SaPI integration has occurred.

Packaging

SaPIs are packaged in particles composed exclusively of phage proteins[17,28]. Packaging requires the conserved SaPI-encoded TerS homologue, presumably complexed with the phage-encoded large terminase subunit, which cleaves the multimeric DNA at the SaPI-specific *pac* site and conducts it to the phage-encoded portal protein for threading into capsids. Filling of the heads is followed by cleavage of the DNA by the large terminase subunit, generating terminally redundant monomeric SaPI DNA, analogous to the DNA of a typical *pac* phage[15].

SaPI1 and SaPIbov1 remodel the phage capsid proteins to generate capsids that are one-third of the size of the helper phage capsids, to accommodate the smaller SaPI genomes but exclude complete helper phage genomes. This requires two SaPI genes, *cp1* and *cp2*, which are adjacent in a LexA-regulated operon called operon I that contains six genes encoding proteins involved in packaging[16] as well as *terS.* The product of *cp2* is present in purified procapsids and is predicted to form a complex with the helper phage scaffolding protein to regulate the size of the capsid[29]. The product of the operon I gene *cp3* seems to affect the amount of SaPI-specific DNA found in small capsids, but whether this protein alters the number of small capsids or affects DNA packaging remains to be determined. The geometry of the typical staphylococcal phage capsomere can accommodate these two capsid sizes; whether other sizes are possible is unknown. of the 16 known SaPIs, 13 are predicted to contain such capsid-remodelling genes (FIG. 1a). one of the exceptions, SaPIbov2, does not produce small capsids[23], in keeping with its much larger genome (~27 kb). The capsid sizes of the other two exceptions, SaPI5 and SaPImw2, have not been examined, but their genomes are in the typical SaPI size range. SaPI DNA packaging is nonspecific; SaPI DNA can be packaged in large and small phage heads, but there is an interesting difference in the relative proportions of these between SaPI and SaPIbov1. SaPI1 DNA is found primarily in small capsids (FIG. 3), whereas SaPIbov1 is packaged into capsids of both sizes with approximately equal efficiency[15]. Furthermore, fragments of helper phage DNA are readily packaged into small capsids during mobilization of SaPI1. SaPIbov1, however, specifically excludes phage DNA from its small capsids[15]. This exclusion depends on a single SaPIbov1 gene, gene 12 (see below).

Interestingly, phage ϕ13, a *cos* phage with a virion genome that contains cohesive termini, can induce excision, circularization and replication of SaPI1 but cannot produce infective SaPI1 particles[20]. This is presumably due to the inability of the ϕ13 *cos* packaging machinery to package SaPI1 DNA. However, phage ϕ11 can package SaPI1 DNA after its excision and replication have been induced by phage ϕ13 (A. Mathews and r.P.N., unpublished observations). Furthermore, phages that cannot induce the SaPI1 excision–replication–packaging cycle, such as phage ϕ147 and phage ϕ85, can package SaPI DNA if excision and replication have been activated by an *stl* mutation[13].

Packaging and transfer of the PrCIs LlCI1 and LlCI2 in *L. lactis*, EfCI538 in *E. faecalis*, and SsuC1 and SpyCIM1 in streptococci have yet to be studied. Some of these lack an identifiable *terS*, and it is not yet known whether they are packaged.

SaPI–phage interactions

In general, SaPI induction results in a substantial decrease of phage reproduction, usually blocking plaque formation. SaPIbov1 gene 12 (*pif*), and, presumably, SaPI1 gene 11 are responsible for this interference and are expressed following induction of the SaPI excision–replication–packaging cycle. Expression of *pif* alone substantially inhibits phage maturation[15]. There are two versions of *pif* among the known SaPI genomes, as exemplified by SaPIbov1 and SaPI1, which contain *pif* genes with around 30% similarity (see Supplementary information S6 (table)). As noted above, *pif* affects packaging specificity as well as phage reproduction. SaPI1 does not exclude phage DNA from its small capsids (although it interferes with phage maturation more strongly than SaPIbov1), nor does it affect phage DNA packaging; in fact, most of the SaPI1 and phage DNAs in mature particles are in small capsids[15].

Trans-species and trans-genera transfer

SaPIbov1 can be transduced to several staphylococci other than *S. aureus*, including Staphylococcus xylosus, Staphylococcus chromogenes, *S. epidermidis*, and *Staphylococcus intermedius*[29]. recent reports have indicated that SaPI1 and SaPIbov1 can be transduced to Listeria monocytogenes (see Supplementary information S7 (table)) but not to streptococci, lactobacilli or Bacillus subtilis[22].

A very high transfer frequency (>10^8 per ml of phage lysate) was observed for SaPI1 in some *L. monocytogenes* strains, and analysis of the resulting transductants suggested that both integrated and autonomous forms were present, indicating that SaPI1 can exist as a plasmid as well as in an integrated form in *L. monocytogenes.* The transfer frequency of a SaPI1 *int* mutant was the same as that of the wild type, and the stability of the transferred SaPI was similar to that of the autonomously replicating SaPI plasmid in *S. aureus* (J. Chen and r.P.N., unpublished observations), indicating that the element can be maintained as a plasmid. Mapping of the integration sites revealed a set of insertion sites that are similar to the secondary *attS* sites in *S. aureus*[22]. (see Supplementary information S3 (table)) Although they could clearly infect *L. monocytogenes*, phage 80α, phage ϕ11 and several other staphylococcal phages did not form plaques on any of the *L. monocytogenes* strains tested[22].

The dramatic finding of SaPI transfer to *L. monocytogenes* has raised the interesting question of whether superantigens expressed by an intracellular pathogen within a eukaryotic host cell are toxic, and it has also been suggested that a novel biohazard will emerge: superantigen-expressing *L. monocytogenes.* Although no naturally-occurring strain of *Listeria* containing any PrCI-like island that produces staphylococcal super-antigens has been identified to date, we consider it highly likely that SaPI transfer to *L. monocytogenes* has already occurred or will soon occur, because both *S. aureus* and *L. monocytogenes* are commonly found in the bovine udder, most bovine and ovine *S. aureus* isolates carry genes for one or more superantigens, and phage therapy of bovine mastitis is increasing[30].

The staphylococcal phages that induce SaPI transfer to *L. monocytogenes* do not form plaques, so silent, phage-mediated transfer may represent a new and important mechanism of horizontal gene transfer among bacteria. Furthermore, given the very high transfer frequency that is observed, this may not be confined to highly mobile units such a SaPIs.

Concluding comments

Studies of the staphylococcal PrCIs — the SaPIs in particular — have revealed the very intimate adaptation of a MGE to the phage life cycle, encoding just those genetic functions that enable the element to use the phage to activate its own replicative machinery and to

provide the structural components of its own specific capsids. we suggest that PrCIs are not defective prophages but, rather, that they have evolved from prophages in a highly specific manner and that they represent a branch of the pathway leading from fully lytic to temperate phages. At the same time, their high mobility places them at one end of the MGE mobility spectrum, and they are a connecting link between prophages and the other MGEs. In the staphylococci, they are the only known repository of several superantigen genes, including *tst*, *seb* and the *sec* genes, and they therefore have a major role in the dissemination of genes that impact both human health and adaptation of the organism to the animal host. They are very common in staphylococci and are probably widespread among other Gram-positive bacteria. Although they have not been described in Gram-negative bacteria or Archaea, they bear some similarity to other MGEs that exploit helper phages, such as satellite phage P4 and Sulfolobus islandicus plasmid pSSVx[31], and it is probably only a matter of time until they are recognized in these groups of organisms as well.

The remaining experimental challenges are, among others: to determine the mechanism of SaPI inhibition of helper phage reproduction; to clarify the relationship between relief of SaPI repression and packaging specificity; to elucidate the nature of the interactions between co-resident SaPIs; to define the molecular genetics and physiology of SaPIs infecting other genera; to determine the bacterial spectrum of PrCIs and their functionality; and to determine the prevalence of PrCI-mediated and phage-mediated silent trans-genera gene transfer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

Prophage	A quiescent form of a bacteriophage (usually inserted into the chromosome of its host), in which the lytic functions of the phage are repressed.
Iteron	One member of a set of short repeated DNA sequences that are located at a bacterial origin of replication and are required for the initiation of replication.
SOS response	A global stress response to DNA-damaging agents such as ultraviolet light or mitomycin C.
Campbell mechanism	A recombinational mechanism for the insertion of a genetic element, such as a phage genome or a plasmid, into the bacterial chromosome, involving circularization of the element followed by a single crossover with a chromosomal target site.
Lysogen	A bacterium containing an inducible prophage.

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Box 1| History of *Staphylococcus aureus* pathogenicity islands

Following the identification of toxic shock syndrome toxin 1 (TSST1)[32], it was observed that the toxin was produced by only 10–20% of natural staphylococcal isolates[33] and that non-producers lacked the gene encoding the toxin (*tst*) plus some 15 kb of additional DNA[34], suggesting that the gene was carried by a mobile genetic element. As chromosomal mapping revealed two different locations for *tst*[35], the mobile element was assumed to be a transposon, and it was designated Tn*557*. However, Southern blotting revealed that the two *tst*-carrying elements were different, leading to their designation as a family of *Staphylococcus aureus* pathogenicity islands (SaPIs)[6].

Using a tetracycline resistance marker in the *tst* gene, the SaPI transduction frequency with staphylococcal phage 80α (but not with the related phage ϕ11) was found to be around 10^7-fold higher than that for the same marker integrated elsewhere in the chromosome. This high frequency was phage specific and recombinase A (RecA) independent. Further, SaPI1 induction interfered strongly with multiplication of the inducing (or helper) phage, phage 80α, blocking plaque formation and reducing the phage burst size by 10-fold to 100-fold, but had no effect on the non-helper phage ϕ11. Finally, SaPI1 DNA was found to be encapsidated in small-headed infective phage-like particles that accommodated the smaller SaPI genome[20] and were composed exclusively of phage virion proteins[17,28].

Box 2| Phage-related chromosomal island nomenclature

The finding of phage-related chromosomal islands (PRCIs) in other genera suggests that such islands are widespread and possibly co-ancestral, and that they require a well-defined nomenclature. We propose that the individual PRCIs are designated with reference to their species — thus, SaPIn, SeCIn, ShCIn, SsCIn, LlCIn, SsuCIn and SpyCIn would be used for *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus*, *Lactococcus lactis*, *Streptococcus suis* and *Streptococcus pyogenes*, respectively, where 'n' refers to a specific island (for example, SsCI15305 for the PRCI identified in the genome sequence of *S. saprophyticus* str. 15305). We further propose to retain the term SaPI rather than change it to SaCI, because of the carriage of virulence factors in this case. It is used in this Review in reference to the *S. aureus* elements. Note that the 'n' in SaPIn1 as previously published[36] refers to *S. aureus* str. n315.

However, the nomenclature of the SaPIs is somewhat controversial. Lindsay *et al.*[6] proposed the SaPIn designation, in which 'n' represents a numerical series. This was adhered to in the description of the first *S. aureus* genome[36] but not in the description of the second one[12]. In this case, a complex classification scheme was proposed instead, using 'ν' as the prefix for most of the chromosomal islands, renaming the SaPIs along with all other putative islands (except for the staphylococcal cassette chromosome *mec* elements (SCC*mec*s), which continued to be listed separately). Lindsay and Holden[37] retained the SaPI designation but changed the specific (numerical) designations to include only those from strains that had been sequenced at the time. They proposed that all SaPI-like elements located at a single chromosomal site be assigned to a single class. We agree with this, but we must point out that the SaPIs have recombined promiscuously[7], resulting in the presence of several diverse elements at single sites. These are listed in TABLE 1 and illustrated in Supplementary information S7 (figure), which should be compared with FIG. 1. A SaPI-like PRCI in the well-known fusidic acid-resistant strain of *S. aureus* was designated SaRIfusB[38] (for *S. aureus* resistance island *fusB*) and has not been renamed.

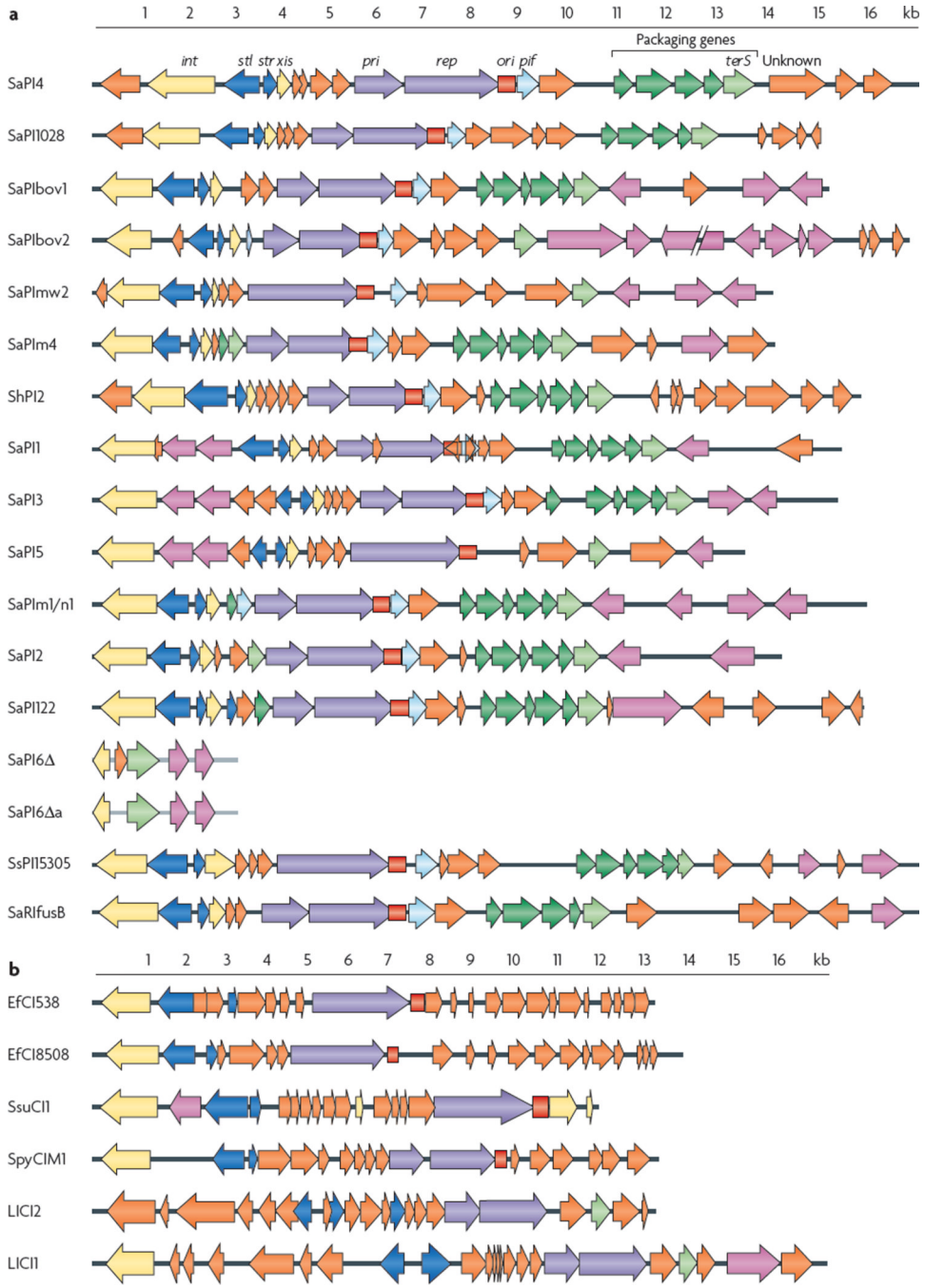

Figure 1. comparison of phage-related chromosomal island genomes

Genomes are aligned according to the prophage convention, with the integrase gene (*int*) at the left end. Genes are coloured according to their sequence and function: *int* and *xis* (excisionase) are yellow; transcription regulators are dark blue; replication genes (including the primase gene (*pri*) and the replication initiator gene (*rep*)) are purple; the replication origin (*ori*) is red; encapsidation genes are green, with the terminase small subunit gene (*terS*) in light green; superantigen and other accessory genes are pink; and *pif* (which functions in phage interference) is light blue. Genes encoding hypothetical proteins are orange. **a** | The gene organization of *Staphylococcus aureus* pathogenicity islands (SaPIs). **b**

| Putative phage-related chromosomal islands from genera other than *Staphylococcus.* SaPIm1/n1 indicates SaPIn1 (from *S. aureus* str. n315) and SaPIm1 (from *S. aureus* str. mu50), which are essentially identical.

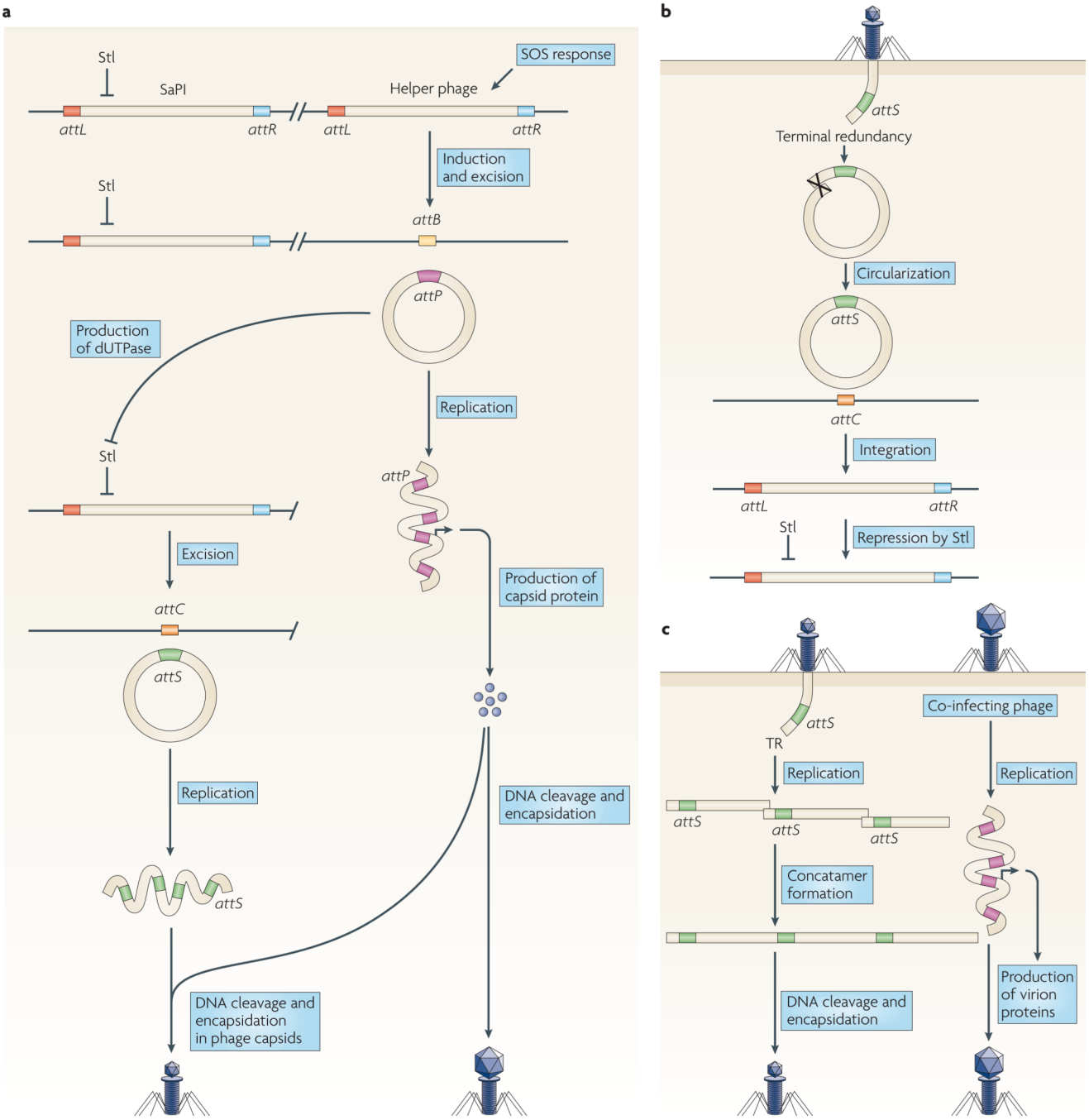

Figure 2. Staphylococcal pathogenicity island replication scenarios

a | *Staphylococcus aureus* pathogenicity island (SaPI) induction by an SOS-induced helper prophage. *attP* and *attS* are the prophage and SaPI core attachment sequences, respectively; *attB* and *attC* are the prophage and SaPI core chromosomal attachment sequences, respectively. After induction and excision of the helper phage, phage dUTPase relieves the Stl-mediated repression of the SaPI, allowing production of SaPI proteins. SaPI excisionase (Xis) subsequently promotes the excision of the SaPI through a Campbell mechanism, restoring the *attC* and *attS* sites. Subsequent SaPI replication produces hundreds of copies in the form of a concatemer, which is cleaved by the terminase complex into individual copies and packed into phage particles consisting entirely of phage proteins but with a smaller head

than the phage capsids, owing to the action of SaPI proteins. **b** | SaPI infection. Terminal redundancy allows the incoming SaPI DNA to circularize, after which the circular DNA is integrated at the chromosomal *attC* site by crossover with the SaPI *attS* site. This process requires the SaPI integrase. Stl silences the expression of SaPI genes, keeping the element integrated. **c** | Co-infection of a SaPI and a helper phage allows the SaPI DNA to be replicated prior to integration. The synthesis of phage proteins leads to the production of phage capsids, which are used to package the replicated SaPI DNA. As in part **a**, SaPI proteins affect the size of the capsids that are produced.

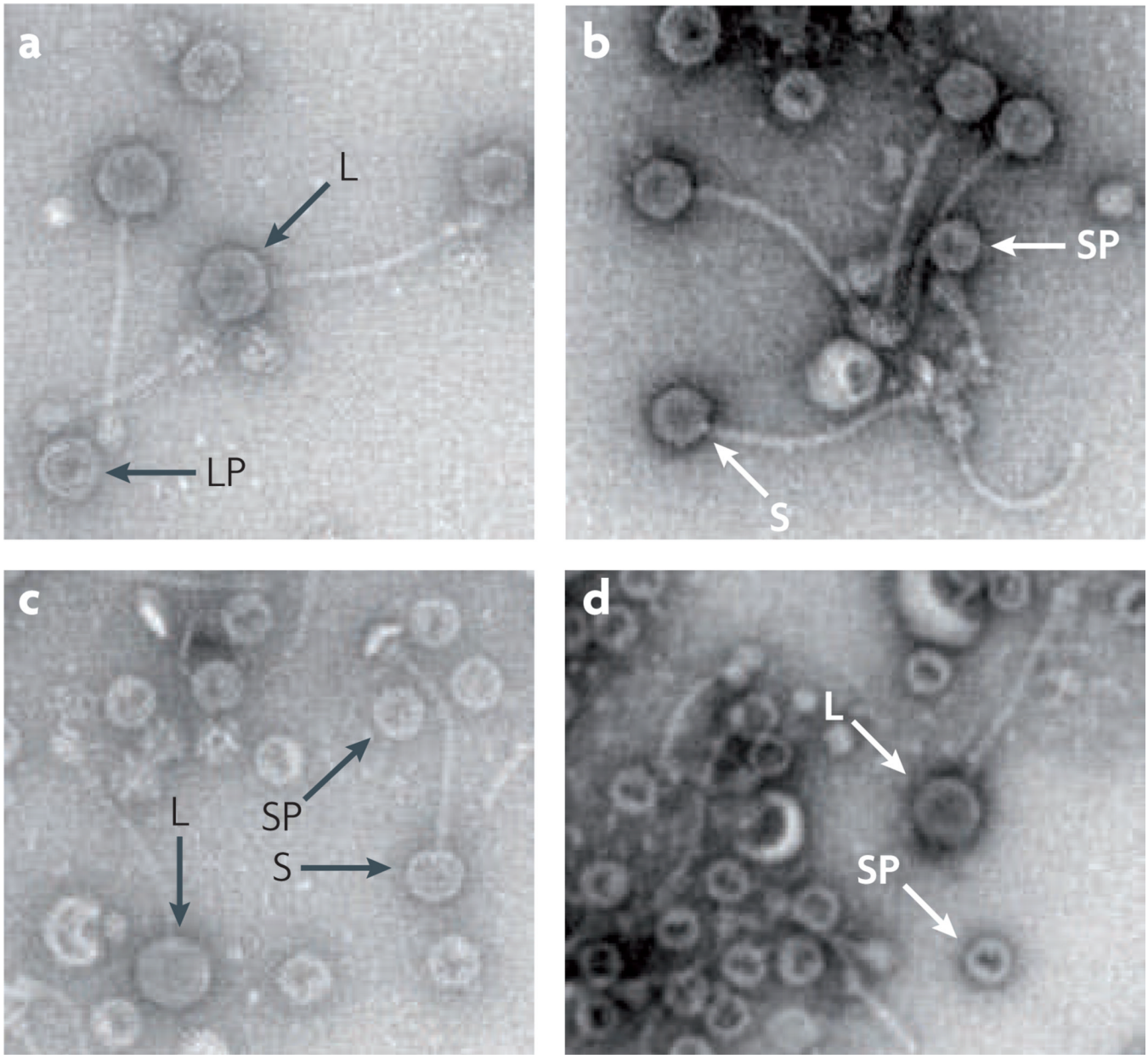

Figure 3. Electron microscopy of phage and *Staphylococcus aureus* pathogenicity island particles

Lysates prepared from *Staphylococcus aureus* strains carrying the relevant *S. aureus* pathogenicity island (SaPI) and/or helper phage were centrifuged, and the pellets were resuspended in buffer, applied to copper grids and negatively stained with uranyl acetate. **a** | Lysate from bacteria carrying phage 80α alone, containing a normal-sized capsids. **b** | Lysate from cells carrying phage 80α and SaPI1. **c** | Lysate from cells carrying phage 80α and SaPI1, showing both the large phage capsids and the small SaPI capsids. **d** | Lysate from cells carrying phage 80α and SaPIbov1Δ*terS*. Despite the absence of TerS, SaPIbov1 induces the formation of small capsids. L, large capsid; LP, large procapsid; S, small capsid; SP, small procapsid.

Table 1

Genes of three *Stapylococcus aureus* pathogenicity island prototypes

ORF number (gene name)*			orientation	Annotation or function
SaPi1	SaPibov1	SaPi2		
attL	*attL*	*attL*	NA	Repeat region
26 (*int*)	21 (*int*)	1 (*int*)	−	Integrase
25	Absent	Absent	−	Hypothetical protein
24 (*sek*)	Absent	Absent	−	Enterotoxin K
23 (*seq*)	Absent	Absent	−	Enterotoxin Q
22 (*stl*)	20 (*stl*)	2 (*stl*)	−	SaPI master repressor
21 (*str*)	19 (*str*)	3 (*str*)	+	Regulatory protein
20	18 (*xis*)	4	+	Excisionase in SaPIbov1
19	17	5	+	Hypothetical protein
Absent	Absent	6	+	Hypothetical protein
18	16	7	+	Hypothetical protein
17 (*pri*)	15 (*pri*)	8 (*pri*)	+	Similar to DNA primase
15 (*rep*)	13 (*rep*)	9 (*rep*)	+	Replication initiator with helicase activity
ori	*ori*	*ori*	+	Replication origin
11 (*pif*)	12 (*pif*)	10 (*pif*)	+	Phage interference
10	11	Absent	+	Hypothetical protein
9	Absent	11	+	Hypothetical protein
Absent	Absent	12	+	Hypothetical protein
8	10	13	+	Hypothetical protein
7 (*cp3*)	9 (*cp3*)	14 (*cp3*)	+	Capsid size determinant
6 (*cp2*)	8 (*cp2*)	15 (*cp2*)	+	Capsid size determinant
5 (*cp1*)	7 (*cp1*)	16 (*cp1*)	+	Capsid size determinant
4	6	17	+	Hypothetical protein
3 (*terS*)	5 (*terS*)	18 (*terS*)	+	Terminase small subunit
2 (*tst*)	4 (*tst*)	19 (*tst*)	−	Toxic shock syndrome toxin 1
1 (*ear*)	Absent	Absent	+	Homologous to penicillin binding protein
Absent	3	Absent	+	Hypothetical protein
Absent	2 (*sec*)	Absent	+	Enterotoxin type C
Absent	1 (*sel*)	Absent	−	Enterotoxin type L
Absent	Absent	20 (*eta*)	−	Similar to *Staphylococcus hyicus* exfoliatin A
attR	*attR*	*attR*	NA	Repeat region

NA, not applicable; SaPI, *Staphylococcus aureus* pathogenicity island;

*SaPI sequences are displayed according to current convention, with *int* at the left end. Some of the early-sequenced SaPIs, including SaPI1 and SaPIbov1, were assigned gene numbers in the opposite orientation.

Table 2

Accessory genes in phage-related chromosomal islands

gene	Product	Function	carried by
tst	Toxic shock syndrome toxin 1 (TSST1)	Superantigen	SaPI1, SaPI2, SaPIbov1, SaPIn1 and SaPIm1
seb	Enterotoxin B	Superantigen	SaPI3
sec	Enterotoxin C	Superantigen	SaPIbov1, SaPIn1, SaPIm1, SaPImw2 and SePI1
ear	Penicillin-biding protein fragment	Penicillin resistance (in *Escherichia coli*)	SaPI1, SaPI3 and SaPI5
sek	Enterotoxin K	Superantigen	SaPI1 and SaPI3
sel	Enterotoxin L	Superantigen	SaPIbov1, SaPI3 and SePI1
seq	Enterotoxin Q	Superantigen	SaPI1 and SaPI5
eta	Exfoliatin A	Epidermolytic toxin	SaPI2
bap	Biofilm-associated protein (BAP)	Biofilm formation in the bovine udder	SaPIbov2
fhuD	Ferrichrome ABC transporter homologue	Iron transport	SaPIm4
mdr	Multidrug resistance protein	Multidrug exporter	SaPI122
aad	Aminoglycoside adenyl transferase	Aminoglycoside resistance	SsPI15305
fosB	Glutathione thionyl phosphatase	Fosfomycin resistance	SsPI15305
fusB	FusB	Fusidic acid resistance	SaRIfusB
ermA	Ribosome methylase	MLS resistance	SpPI1
Unnamed	Unnamed	Phage resistance	LlCI1
actA	GNAT family homologue	Acetyl transfer protein	SaPI6Δ
None	NA	NA	SaPI4, SaPI1028, ShPI2 and LlCI2

ABC, ATP-binding cassette; MLS, macrolide–lincosamide–streptogramin B; NA, not applicable; SaPI, *Staphylococcus aureus* pathogenicity island.

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Table 3

The staphylococcal pathogenicity island family

Element	Staphylococcal genome	Baba*	Lindsay and Holden‡	Size (kb)	Inducing phages	*att* site core (location, *att*/*int* group)	Refs
SaPI4	*S. aureus* str. MRSA252	NA	SaPI4	15.1	Endogenous prophage	AAAGAAGAACAATAATAT (~8′, I)	7,39
SaPI1028	*S. aureus* str. NY940	NA	NA	15.6	Endogenous prophage	AAAGAAGAACAATAATAT (~8′, I)	7,40
SaPIbov1	*S. aureus* str. RF122	νSa2	NA	15.8	φ11 and 80α	TAATTATTCCCACTCAAT (~9′, II)	25,41
SaPIbov2	*S. aureus* str. V329	NA	NA	27	80α	TAATTATTCCCACTCGAT (~9′, II)	25
SaPIm4	*S. aureus* str. mu50	νSa3 type I	NA	14.4	Endogenous prophage	TCCCGCCGTCTCCAT (~18′, III)	7,12
SaPImw2	*S. aureus* str. mw2	νSa3 type II	SaPI3	14.4	Endogenous prophage	TCCCGCCGTCTCCAT (~18′, III)	7,12
SePI1	*S. aureus* str. FRI909	NA	NA	9.9	Not known	TCCCGCCGTCTCCAT (location unknown§, III)	11
ShPI2	*S. haemolyticus*	νSh2	NA	16.6	Not known	TCCCGCCGTCTCCAT (48′, III)//	8
SaPI1	*S. aureus* str. RN4282	νSa1	NA	15.2	80α and φ13	TTATTTAGCAGGAATAA (~19′, IV)	6
SaPI3	*S. aureus* str. COL	νSa1	SaPI1	15.6	Not known	TTATTTAGCAGGAATAA (~19′, IV)	42
SaPI5	*S. aureus* str. USA300	NA	NA	14.0	Not known	TTATTTAGCAGGAATAA (~19′, IV)	43
SaPIn1 and SaPIm1	*S. aureus* str. n315 and *S. aureus* str. mu50, respectively	νSa4 type I	SaPI2	15	80α	GTTTTACCATCATTCCCGGCAT (~44′, V)	36 and J.R.P., unpublished observations
SaPI2	*S. aureus* str. RN3984	NA	NA	14.7	80 and 80α	ATTTTACATCATTCCTGGCAT (~44′, V)	7,20
SaRIfusB	*S. aureus* European fusidic acid-resistant impetigo clone CS6	NA	NA	20.7	Not known	ATGCCAGGTATGATGTAAAAC (~44′, V)	38
SaPI122	*S. aureus* str. RF122	NA	NA	17.9	Endogenous prophage	GTTTTACATCATTCCTGGCAT (~44′, V)	NA¶
SaPI6Δ	*S. aureus* strains 8325, COL, USA300, MSSA476, Newman and mw2	νSa4 type II	NA	3.14	Not known	GTTTTACCATCATTCCCGGCAT,GTTTTA CATCATTCCTGGCAT (~44′, V)	12
SsPI15305	*S. saprophyticus str.* 15305	νSs15305	NA	16.7	Not known	Unknown sequence (~48′, VI)	9

int, integrase; NA, not applicable; *S. aureus*, *Staphylococcus aureus*; *S. haemolyticus*, *Staphylococcus haemolyticus*; *S. saprophyticus*, *Staphylococcus saprophyticus*.

*Nomenclature proposed by Baba *et al.*[36].

[≠] Nomenclature used by Lindsay and Holden[37].

[§] This strain has not been sequenced yet, so the genomic location of SaPI1 is unknown.

[//] ShPI2 is located 180° away from the other SaPIs with the same *att* core sequence, owing to the major chromosomal inversion that has been documented in the *S. haemolyticus* genome[8].

[¶] GenBank accession NC_007622.