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Staphylococcal Plasmids, Transposable and Integrative Elements

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ABSTRACT Strains of *Staphylococcus aureus*, and to a lesser extent other staphylococcal species, are a significant cause of morbidity and mortality. An important factor in the notoriety of these organisms stems from their frequent resistance to many antimicrobial agents used for chemotherapy. This review catalogues the variety of mobile genetic elements that have been identified in staphylococci, with a primary focus on those associated with the recruitment and spread of antimicrobial resistance genes. These include plasmids, transposable elements such as insertion sequences and transposons, and integrative elements including ICE and SCC elements. In concert, these diverse entities facilitate the intra- and inter-cellular gene mobility that enables horizontal genetic exchange, and have also been found to play additional roles in modulating gene expression and genome rearrangement.

As has been the case for other bacterial genera, studies of the genetic basis of staphylococcal pathogenicity and antimicrobial resistance highlighted the presence of determinants not uniformly represented in the genomes of all staphylococcal strains. Although not a fundamental requirement for survival *per se*, such accessory DNA usually encodes functions that are advantageous in a particular environmental niche. The extent of the accessory component of the genome has been revealed by comparative analysis of whole *Staphylococcus aureus* genome sequences, which indicate that it can constitute in excess of 20% of a strain's genetic makeup (1–5). It is clear that the acquisition, maintenance, and dissemination of accessory functions have been central to the ongoing success of staphylococci as pathogens. These processes are underpinned by interactions between mobile genetic elements, such as insertion sequences and transposons that mediate intracellular movement of DNA, and plasmids, bacteriophage and integrative and conjugative elements (ICEs) that facilitate intercellular DNA mobility.

MECHANISMS OF GENETIC EXCHANGE

DNA can be introduced into staphylococci by each of the three classical bacterial gene transfer mechanisms of transformation, transduction, and conjugation. Trans-

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formation is thought to be limited by extracellular nucleases and is usually very inefficient (6, 7). However, homologs of competence genes from other Gram-positive species are evident in the *S. aureus* chromosome, leading to the suggestion that natural competence might arise under suitable growth conditions and/or in subpopulations of cells (8). Staphylococci usually possess one or more prophages integrated within their genomes, and generalized transduction is likely to be a frequent mechanism of genetic exchange between strains (9, 10). Staphylococcal conjugative plasmids not only mediate their own self-transfer, but also facilitate the exchange of other coresident nonconjugative plasmids, either by mobilization if the other plasmid carries a relaxation system (11) or more generally via cointegrate formation, and potentially subsequent resolution, in a process termed "conduction" (12, 13). Conjugation is also thought to represent the most likely basis for the apparent transmission of large DNA segments that correspond to approximately 10 to 20% of the chromosome (14). In addition to their own transfer, chromosomally located ICEs in staphylococcal strains (15) might also mediate transmission of plasmid and chromosomal DNA (16). A novel but poorly understood mechanism of staphylococcal genetic exchange, termed mixed-culture transfer or phage-mediated conjugation, was described in the 1980s (6, 17, 18). Although phage, or perhaps components of phage, play a role, the process has been shown to be mechanistically distinct from transduction. More recently, another phage-mediated mechanism, termed autotransduction, has been defined (19), which like mixed-culture transfer, requires the DNA recipient to be lysogenic for specific prophage. It is possible that mixed-culture transfer and autotransduction are actually different perspectives on the same phenomenon. There have been several reports that describe enhanced DNA transfer between staphylococci, by conjugation and transduction, in response to subinhibitory levels of some antibiotics; however, in most cases the basis of this stimulation is yet to be delineated (10, 20–22). Notably, genetic exchange has also been observed to occur more efficiently *in vivo* than *in vitro* (23).

Identical or nearly identical accessory genes, elements, and plasmids have been detected in different staphylococcal species and other bacterial genera, such as enterococci and streptococci (24–28). Such observations suggest that, directly and/or indirectly, the gene transfer mechanisms operating in staphylococci facilitate not only intraspecific transfer, but also interspecific and intergeneric exchange and hence access to an extended and shared reservoir of determinants.

STAPHYLOCOCCAL PLASMIDS

One or more plasmids are usually found in clinical isolates of *S. aureus* and coagulase-negative staphylococci (7, 29). Historically, staphylococcal plasmids can be categorized into one of three main classes based on physical/genetic organization and functional characteristics (24, 25, 30). These correspond to small plasmids that utilize a rolling circle (RC) replication mechanism and usually carry a single resistance gene or are cryptic, larger multiresistance plasmids that often carry several resistance genes and conjugative plasmids that usually also contain multiple resistance determinants and are typically larger, again due to the conjugation-associated genes they contain; theta-mode replication is used by multiresistance and conjugative plasmids. However, categorization based on resistance genes is parochial and does not necessarily reflect relatedness between plasmids, since resistance determinants can be readily lost from, or acquired by, plasmid backbones. For example, there are plasmids closely related to multiresistance and conjugative plasmids that lack any recognized resistance genes. Toxins are also encoded by some plasmids (29, 31–34), and the functions of many genes carried by larger plasmids have not been investigated, let alone elucidated.

Analysis of fully sequenced *S. aureus* plasmids in the databases has illustrated that plasmid size correlates strongly with the mode of replication used and hence with the replication initiation gene possessed. Plasmids smaller than 10 kb use the RC mechanism, whereas those larger than 14.5 kb utilize theta-mode replication, with plasmids in the intermediate 10 to 14.5-kb size range using either of these mechanisms (35). Fifteen plasmid incompatibility groups (Inc1 to Inc15) were established for staphylococcal plasmids, incorporating both RC replicating (RCR) and theta replicating plasmids (24, 36). However, the rate of plasmid discovery through genome sequencing has rendered incompatibility testing impractical. A plasmid typing system for plasmids from Gram-positive bacteria (including staphylococci) based on replication region DNA sequence has been established (37) and incorporated into the PlasmidFinder database (38). Unfortunately, coincident identification of additional replicon types (39, 40) has subsequently resulted in discordant classification of some staphylococcal plasmids.

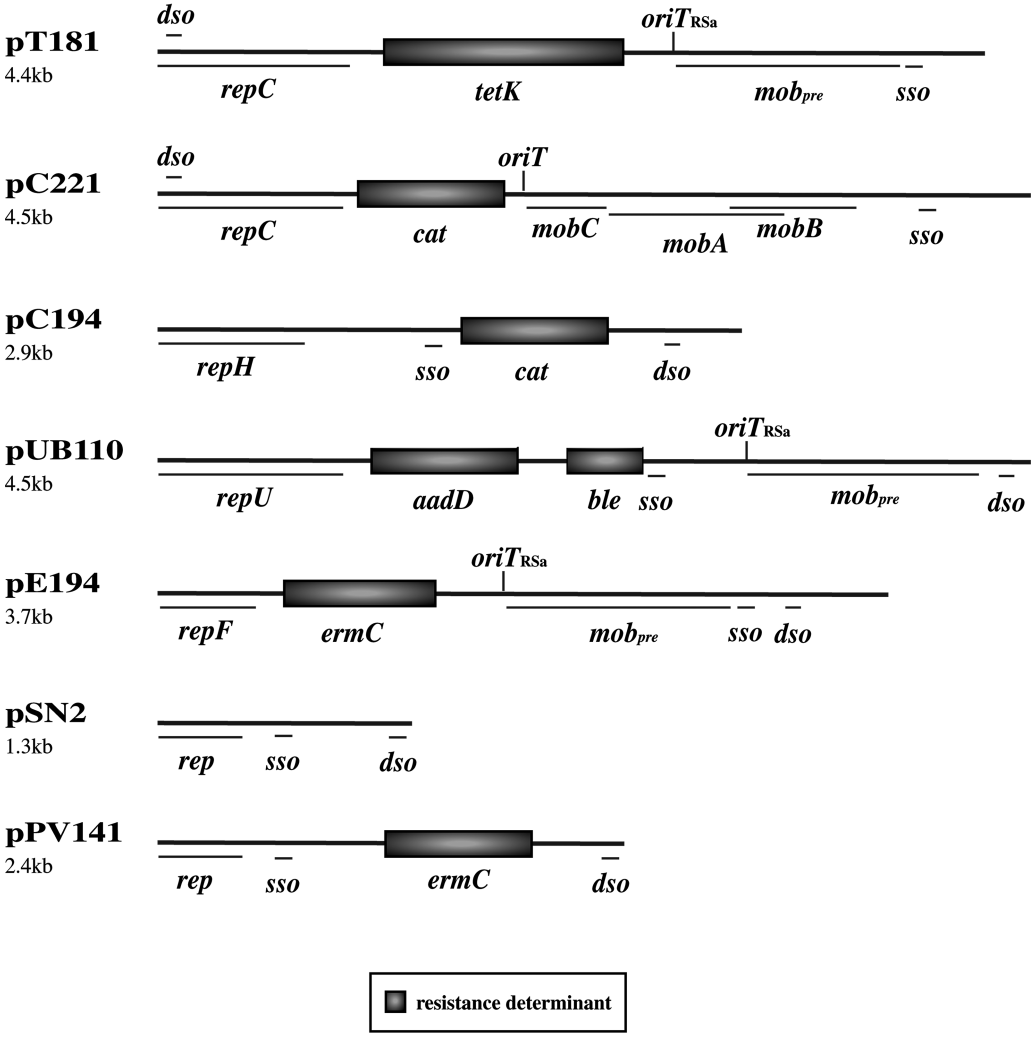
Small RCR Plasmids

The most thoroughly characterized staphylococcal plasmids are those that utilize asymmetric RC replication via a single-stranded DNA intermediate (for detailed

reviews of RC plasmid replication, see Khan [41] and Ruiz-Maso et al. [44]). Probably reflecting constraints imposed by this replication strategy, RCR (sometimes alternatively called just RC or single-stranded DNA) plasmids are usually less than 5 kb in size and are rarely found to contain transposable elements (24). This restriction in RCR plasmid size probably reflects elevated production of high-molecular-weight DNA with increasing plasmid size, which impairs cell growth, leading to counterselection against host cells and hence the plasmid itself (42). RCR plasmids are frequently found cointegrated within larger theta replicating plasmids, commonly flanked by insertion sequences such as IS*257*; in such cases the replication system of the integrated RCR plasmid is often inactivated in some way (28, 43).

Staphylococcal RCR plasmids can be subdivided into four families, exemplified by pT181, pC194, pE194, and pSN2, based on replication region sequence similarity (24); representative plasmids are shown in Fig. 1. These families contain plasmids found in different staphylococcal species and are related to plasmids of other bacterial genera, such as *Bacillus*, *Lactobacillus*, and *Streptococcus*, attesting to the horizontal transmission

FIGURE 1 Maps of representative RCR plasmids: pT181, pC221, pC194, pUB110, pE194, pSN2, and pPV141 (25, 231); see text for additional references. Plasmid sizes are shown on the left. Resistance and plasmid maintenance genes/loci are shown: *rep*/*repC*/*repF*/*repH*/*repU*, initiation of replication; *dso*, double-stranded origin of DNA replication; *sso*, single-stranded origin of DNA replication; *mobA*/*mobB*/*mobC*/mob_{pre}, mobilization; *oriT*/$oriT_{RSa}$, origin of DNA transfer; *cat*, chloramphenicol resistance. Refer to Tables 1 and 2 for the antimicrobial resistance(s) conferred by other resistance determinants.



of RCR plasmids (42). Plasmids belonging to each of these four families encode evolutionarily distinct replication initiator (Rep) proteins that possess the conserved domains Rep_trans, Rep_1, Rep_2, and RepL, respectively (35). However, despite the Rep proteins having unique evolutionary origins, they perform a remarkably similar role in recognizing, binding, and nicking specific DNA sequences in the double-strand origin (*dso*). In the RCR plasmids examined so far, the Rep proteins also perform cleavage and ligation reactions at the termination of leading strand synthesis (44). pT181 family Rep proteins recruit the host-encoded helicase, PcrA, which is essential for synthesis of the new, and displacement of the parental, leading strands (45, 46). The leading strand contains a single-strand origin of replication (*sso*) that, upon displacement, is recognized and primed for replication by host-encoded RNA polymerase (47). The inability of host RNA polymerase to recognize and prime the *sso* limits the host range of RCR plasmids. For example, the *ssoA* carried by pT181 only functions efficiently in staphylococci, whereas the *ssoU* carried by pE194 family plasmids allows stable replication in a broader range of Gram-positive hosts (48).

RCR plasmids are usually maintained at 10 to 60 copies per cell (7), and copy number control is achieved via an antisense RNA that is transcribed divergently to the replication initiation gene (35). In the case of pT181, binding of the antisense RNA causes transcriptional attenuation of *repC* mRNA (49), whereas for pC194 family plasmids antisense binding is believed to block translation of the Rep protein (50, 51). Antisense inhibition of Rep translation is also thought to occur in pE194 family plasmids, but transcriptional regulation mediated by a repressor protein may also play a role (35), as has been shown for the streptococcal RCR plasmid pMV158 (52, 53).

Some staphylococcal RCR plasmids contain mobilization systems that facilitate their horizontal transmission when coresident with a conjugative plasmid. Plasmids such as pC221 possess a multigene mobilization system (*mobCAB* and origin of transfer [*oriT*]) (Fig. 1) (11). The pC221 MobA relaxase and MobC relaxase accessory proteins are both required for nicking at *oriT* (54, 55). Other RCR plasmids possess a different mobilization locus that is homologous to the *mobM* relaxase gene and *oriT* of pMV158 (56, 57). This locus was originally identified as a site-specific recombination function on staphylococcal plasmids such as pT181 and pE194 (Fig. 1) (24), where the constituent sequences were named *pre* and RS_A (corresponding to mob_{pre} and $oriT_{RSa}$, respectively) (Fig. 1). Additionally, the replication initiation protein and *dso* of several RCR plasmids have been shown to "moonlight" as a relaxase and *oriT* for mobilization mediated by the ICE Tn*916* (see below) (58).

RCR plasmids, including those in different families, often share highly similar DNA segments, such that they appear to be mosaic structures consisting of discrete functional cassettes encoding replication, resistance, and sometimes recombination/mobilization functions (24, 59). Exchange of these segments between plasmids is thought to be promoted by the single-stranded intermediates generated by the RC replication strategy, which enhance the frequency of homologous recombination events between regions of flanking sequence similarity (60). DNA processing at replication and transfer origins also promotes the swapping of such cassettes (24, 61).

Theta Replicating Plasmids

Most staphylococcal plasmids larger than 10 kb employ theta-mode replication and are maintained at low copy numbers (7, 35, 62–65). The majority encode a replication initiation protein containing an N-terminal conserved RepA_N domain (29, 35, 66), which corresponds to a winged helix-turn-helix DNA binding domain that binds to origin Rep box repeat sequences characteristically located centrally within the *rep* gene coding sequence (67–69); the C-terminal domain of the Rep protein from the *S. aureus* conjugative plasmid pSK41 (see below) has been shown to interact with the host encoded DnaG primase (70). An interesting variation is seen in the plasmid pTZ2162, where the RepA_N-type initiation protein has been split into two smaller distinct polypeptides, expressed from two partially overlapping *rep* genes that have likely arisen from a frameshift (71). In staphylococcal plasmids encoding RepA_N type initiators, copy number is thought to be controlled by a small antisense RNA that binds to the leader region of the *rep* mRNA to block its translation (68, 72, 73).

Two groups of nonconjugative multiresistance plasmids were described previously in staphylococci: the β-lactamase/heavy-metal resistance plasmids and the pSK1 family plasmids; both utilize RepA_N initiation proteins (74). *S. aureus* strains from the 1960s and 1970s commonly contained plasmids that conferred resistance to β-lactam antibiotics and heavy metals or other inorganic ions. These plasmids characteristically carried a Tn*552*-like β-lactamase-encoding transposon or a derivative thereof (75, 76) and frequently possessed a composite structure designated Tn*4004* conferring

resistance to mercuric ions and operons encoding resistance to arsenical and/or cadmium ions (Fig. 2) (6, 77). Additionally, some β-lactamase/heavy-metal resistance plasmids carry the transposon Tn*4001* or Tn*551* conferring resistance to aminoglycosides (6, 78) and macrolide-lincosamide-streptogramin type B (MLS) antibiotics (79), respectively, and/or a *qacA* or *qacB* gene mediating multidrug resistance to antiseptics and disinfectants (Fig. 2) (6).

Plasmids related to the prototype, pSK1 (Fig. 2), were first identified in epidemic *S. aureus* and coagulase-negative staphylococcal strains isolated in Australia during the 1980s and later in isolates from Europe (25). In addition to a *qacA* gene mediating multidrug resistance to antiseptics/disinfectants carried ubiquitously by pSK1 family plasmids (80, 81), members of this family variously encode Tn*4001*, which confers resistance to gentamicin and other aminoglycosides (82), a Tn*552*-like β-lactamase transposon, Tn*4002* (83), and/or a composite structure designated Tn*4003* that mediates trimethoprim resistance (84). Tn*4003* represents a vestige of a cointegrated pSK639-like plasmid (see below (26, 85).

As more plasmid sequences have accumulated through genome sequencing it has become clear that there is a broad diversity of plasmids with a *repA_N*-type initiation gene, including plasmids lacking resistance genes and/or containing toxin genes, such as pIB485 (Fig. 2) and related plasmids that have been isolated from strains of wide geographic distribution (29). Two other loci are nearly ubiquitous on RepA_N-encoding plasmids, suggesting that they are important for plasmid survival (29). The first is a multimer resolution system that includes a gene coding for a serine recombinase, which is usually annotated as *sin* or *bin3* (86, 87). The second is homologous to the pSK1 *par* (formerly *orf245*) gene, which is usually located upstream of, and transcribed divergently from, the *rep* gene. This locus increases plasmid segregational stability and is thought to constitute a novel single-protein partitioning system (64, 88). In contrast, type I and II partitioning systems, comprising two protein-encoding genes, are found only occasionally on these plasmids (29). Finally, an Fst-like type I toxin-antitoxin system, which might contribute to plasmid segregational stability, is carried by pSK1 (89), and related loci have been identified on numerous other theta replicating staphylococcal plasmids (90, 91).

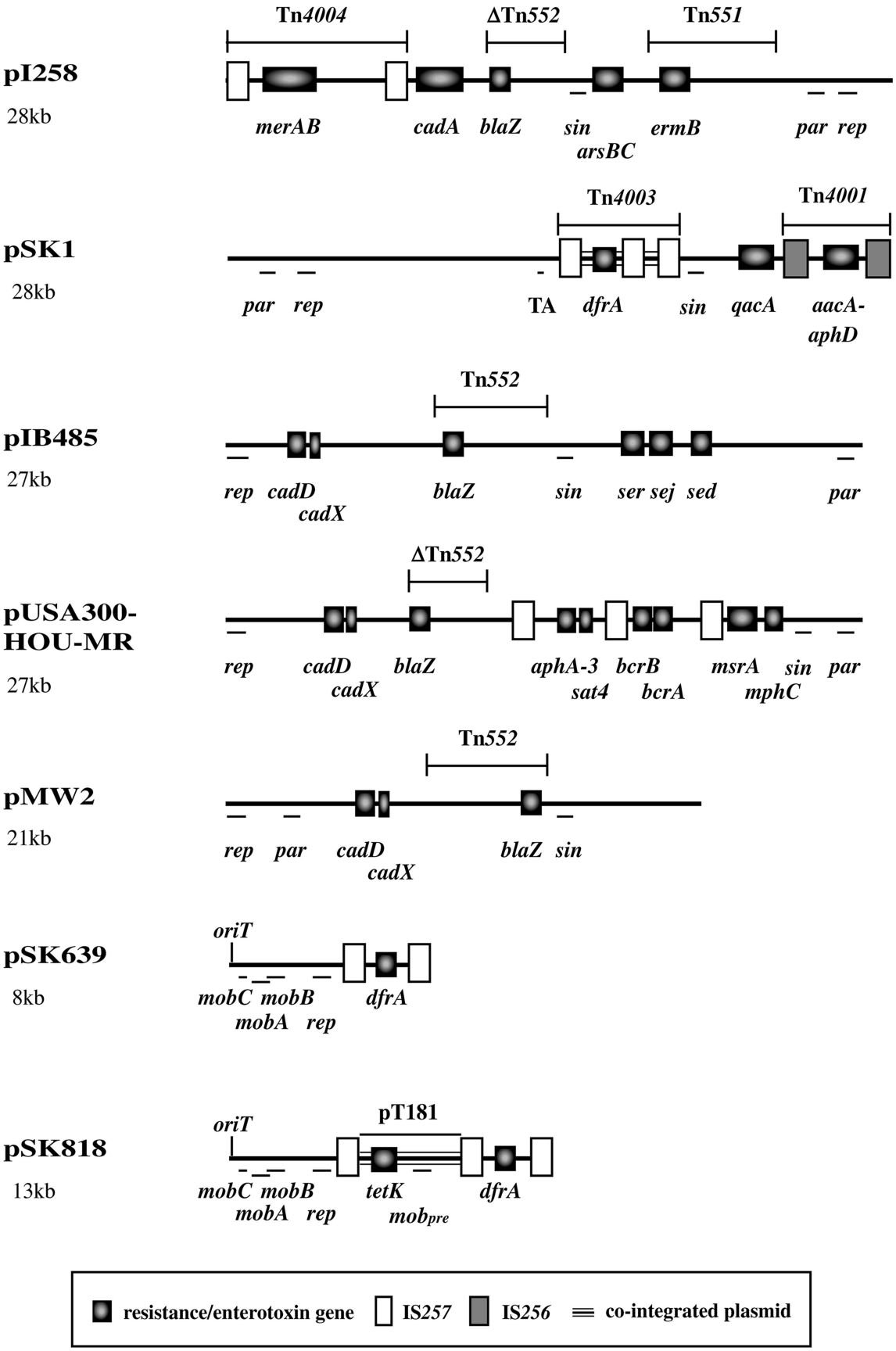
A minority of theta replicating staphylococcal plasmids code for a Rep protein containing a Rep_3 conserved domain (35); initiation proteins of this type from Gram-negative bacteria, encoded by iteron plasmids such as F, pPS10, and R6K, contain tandem winged helix-turn-helix domains (92). This type of replication system was first detected in the genus in plasmids from *Staphylococcus epidermidis* related to the 8-kb trimethoprim resistance plasmid pSK639 (Fig. 2), which also contains a mobilization system related to that of the RCR plasmid pC221 (65). More recently, *rep_3*-type genes have been found in addition to the usual *repA_N*-type gene on larger β-lactamase/heavy-metal resistance plasmids such as pN315 (1), whereas in the case of pMW2 (3) (Fig. 2), only a remnant of the *repA_N* gene remains. Finally, plasmids employing an initiation protein containing a PriCT_1 conserved domain, such as pETB, which encodes exfoliative toxin B (32), have also been found in staphylococci but appear to be quite rare; some also encode a RepA_N or Rep_3 type initiator (35).

Conjugative Plasmids

The largest staphylococcal plasmids (>30 kb) are those that encode their own conjugative transfer (7, 25). Three distinct families of conjugative plasmids have been identified in staphylococci: the well-known pSK41/pGO1-like plasmids and the recently characterized pWBG4 and pWBG749 families.

S. aureus plasmids such as pSK41 (43, 93, 94) (Fig. 3), pGO1 (95, 96), and pJE1 (20) were first identified in association with gentamicin resistance in the mid 1970s (13, 25). Structurally related conjugative plasmids were found in coagulase-negative staphylococci (97, 98), and interspecific transfer has been demonstrated (99, 100). In pSK41 and many relatives, the plasmid backbone consists of two IS*257*-flanked segments, termed the transfer (*tra*) region and region I (Fig. 3) (94). However, in other family members (e.g., pPR9) these segments are contiguous, and this likely represents the ancestral configuration prior to the incorporation of additional DNA segments (43, 101, 102).

Multiple copies of IS*257*, mostly in the same orientation, are a feature of many pSK41-like plasmids (Fig. 3). These usually delimit segments that carry resistance genes and often correspond to cointegrated copies of smaller plasmids (43, 103). Such determinants include the aminoglycoside and bleomycin resistance genes *aadD* and *ble*, respectively, on a copy of pUB110 and the small multidrug resistance determinant *qacC* on another RCR plasmid (Fig. 3). Plasmids such as pJE1 and pGO1 also contain a Tn*4003*-like structure derived from a pSK639-like trimethoprim-resistance plasmid (see above). The *aacA-aphD* gene encoded by Tn*4001*-IS*257* hybrid structures is responsible for resistance to gentamicin and other aminoglycosides (104). Members of the family


pI258
28kb
Tn4004
ΔTn552
Tn551
merAB
cadA
blaZ
sin
arsBC
ermB
par
rep
pSK1
28kb
Tn4003
Tn4001
par
rep
TA
dfrA
sin
qacA
aacA-
aphD
pIB485
27kb
Tn552
rep
cadD
cadX
blaZ
sin
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sed
par
pUSA300-
HOU-MR
27kb
ΔTn552
rep
cadD
cadX
blaZ
aphA-3
sat4
bcrB
bcrA
msrA
mphC
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pMW2
21kb
Tn552
rep
par
cadD
cadX
blaZ
sin
pSK639
8kb
oriT
mobC
mobA
mobB
rep
dfrA
pSK818
13kb
oriT
pT181
mobC
mobA
mobB
rep
tetK
mobpre
dfrA
resistance/enterotoxin gene
IS257
IS256
co-integrated plasmid

have also been found that contain IS*257*-flanked genes encoding resistance to tetracycline (*tetK*) (29), MLS antibiotics (*ermC*) (105), mupirocin (*mupA/ileS2*) (102, 106), and most recently, multidrug resistance to phenicols, lincosamides, oxazolidinones (e.g., linezolid), pleuromutilins, and streptogramin A compounds ($PhLOPS_A$ phenotype; *cfr*) (107). Plasmids of this type can also carry unit transposons, such as Tn*552* (*blaZ*) (43) and the *vanA* glycopeptide resistance transposon Tn*1546* (27). The pSK41-like plasmid pLW1043 (Fig. 3) was responsible for the first reported high-level vancomycin-resistant *S. aureus* strain, isolated in Michigan in 2002 (27), and resulted from the transposition of Tn*1546* into a copy of IS*257* within the pSK41-like plasmid pAM829 (27, 108). A Tn*1546*-containing Inc18 broad-host-range conjugative plasmid, pAM830, carried by a coisolated *Enterococcus faecalis* strain, is thought to have mediated the intergeneric transfer event (27, 109). Such enterococcal Inc18 *vanA* plasmids themselves have subsequently been detected in some vancomycin-resistant *S. aureus* strains, but their scarcity in *S. aureus* suggests some limitation on their establishment in this species, possibly inefficient replication, an incompatibility with staphylococcal cell wall biosynthesis, a restriction barrier, and/or high metabolic burden associated with *vanA* carriage (108). Cointegrates composed of Inc18 and pSK41-like plasmids are also thought to occur (110), and it has been suggested that carriage of pSK41-like plasmids might enhance the recipient ability of *S. aureus* cells for enterococcal donors containing Inc18 plasmids, but the mechanism has not been determined (111).

Conjugative transfer of pSK41 family plasmids occurs only on solid surfaces and does so at comparatively low frequencies, in the range of 10^{-5} to 10^{-7} transconjugants per donor cell (13). The majority of genes thought to be responsible for conjugative transfer are located in the *tra* region (Fig. 3) (93, 95). The *tra* genes are most similar to the transfer genes of the *Lactococcus lactis* plasmid pMRC01 (112) and the broad host range plasmid pIP501 (43), originally identified in *Streptococcus agalactiae*, exhibiting both synteny and product amino acid sequence similarity (43, 113, 114). Several deduced products show homology to proteins from the type IV secretion system superfamily, including to coupling proteins, peptidoglycan hydrolases, and several other core mating pair formation-type proteins (115, 116). Thus, the basic conjugative process is likely to resemble that of more extensively characterized Gram-negative counterparts, *viz.*, transfer of a single strand of plasmid DNA via direct cell-cell contact. However, no pilus-like structure seems to be associated with Gram-positive conjugation (114), perhaps reflecting the distinction in the cell envelope organizations and accounting for the solid surface requirement for conjugative transfer. The origin of transfer, *oriT*, and the relaxase, Nes, that acts upon it, are encoded in region I (117, 118), as are a number of genes of unknown function that might also play a role in conjugative transfer (20, 43, 113).

As mentioned above, pSK41-like conjugative plasmids encode a RepA_N type replication initiation protein, whose translation is regulated by an antisense RNA called RNAI (67, 70, 72, 73). However, several members of the family, such as pPR9 and pUSA03, possess a novel chimeric *rep* gene where the sequences that usually encode the N-terminal RepA_N DNA-binding domain, and the origin Rep box repeats to which it binds, appear to have been replaced by sequences encoding a helix-turn-helix protein sequence related to phage replication proteins and distinct DNA sequence repeats (35, 102). The usual upstream antisense RNA control region and downstream in-frame end of the *rep* gene, encoding the conserved C-terminal end of the Rep protein that interacts with host DnaG primase (70), remain intact in these plasmids, but the variation is sufficient to allow compatibility with the pSK41 replication system (102).

All known pSK41 family plasmids encode a serine recombinase and a type II active partitioning system, the latter representing a significant difference from *repA_N* replicating nonconjugative staphylococcal plasmids, which usually contain an unusual *par* gene or occasionally a type I partitioning system (see above). The pSK41 *res* locus is functional, facilitating the conversion

FIGURE 2 Maps of representative nonconjugative theta-replicating plasmids: pI258, pSK1, pIB485, pUSA300-HOU-MR, pMW2, pSK639, and pSK818 (3, 25, 29, 65, 85, 89, 232); see text for additional references. Resistance/enterotoxin genes, transposons, insertion sequences, and cointegrated plasmids are shown: *arsBC*, arsenic resistance; *cadA*, cadmium resistance; *mphC*, macrolide resistance; *msrA*, macrolide/streptogramin B resistance; *qacA*, antiseptic/disinfectant resistance; *ser*, *sej*, and *sed*, enterotoxins. Refer to Tables 1 and 2 for the antimicrobial resistance(s) conferred by other resistance determinants. Plasmid maintenance genes/loci are also shown: *par*, novel partitioning system; *rep*, initiation of replication; *sin*, multimer resolution; TA, Fst-like toxin-antitoxin system; *mobA/mobB/mobC/*mob_{pre}, mobilization.

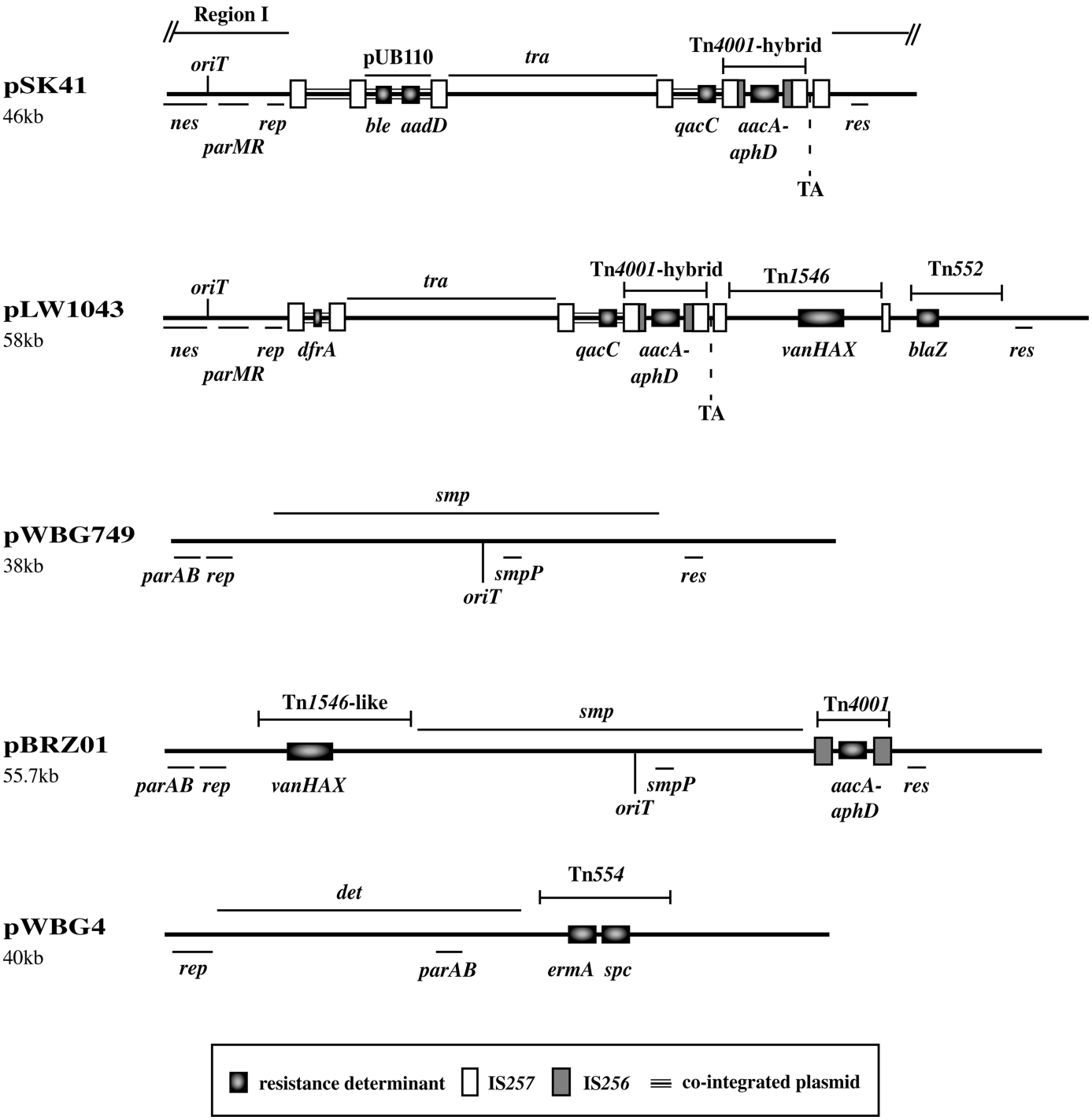


FIGURE 3 Maps of representative conjugative theta-replicating plasmids: pSK41, pLW1043, pWBG749, pBRZ01, and pWBG4 (27, 43, 128, 136, 137); see text for additional references. Resistance genes, transposons, insertion sequences, and cointegrated plasmids are shown: refer to Tables 1 and 2 for the antimicrobial resistance(s) conferred by the resistance determinants. Plasmid maintenance genes/loci (if known) are also shown: *parAB*, type I partitioning system; *parMR*, type II partitioning system; *rep*, initiation of replication; *res*, multimer resolution; TA, Fst-like toxin-antitoxin system; *tra*/*smp*/*det* genes, conjugative transfer; *nes*/*smpP* genes, relaxases; *oriT*, origin of transfer. Note that pLW1043 and pBRZ01 are members of the pSK41 and pWBG749 families, respectively.

of plasmid multimers into monomers, with the Res protein shown to bind to a resolution site, comprising three subsites, located in the intergenic region upstream of the *res* gene; Res binding also autoregulates transcription of its own promoter (119). Functionality of the pSK41 partitioning system has also been demonstrated (120). It comprises a centromere-like site, *parC*, and two genes in an operon, *parM* and *parR*. The ParM protein is an actin-like motor protein that forms single-stranded helical filaments (121). ParC is a ribbon-helix-helix protein that binds to DNA repeat sequences within *parC* (120) and interacts with ParM to modulate its activity (122). ParR also autoregulates transcription from the partitioning operon promoter, which is also controlled by the plasmid-encoded ArtA protein (see below). Some pSK41-like plasmids encode an additional potential plasmid maintenance determinant in the form of an Fst-like toxin-antitoxin system, which is located on a small IS*257*-flanked DNA segment adjacent to Tn*4001*-hybrid elements in plasmids conferring gentamicin resistance (94).

In pSK41, transcription of the majority (21 out of 30) of the plasmid backbone genes is controlled by the product of the conserved *artA* gene (corresponding to *trsN* on pGO1) (101, 123). The ArtA protein is a ribbon-helix-helix DNA-binding protein that represses five promoters in addition to its own—including two operon promoters in the *tra* region and two more operon promoters in region 1 (Fig. 3). ArtA is therefore thought to coordinate basal-level transcription of most of the plasmid's backbone genes (101).

The pWBG4 family of conjugative plasmids, also called diffusible pigment (DiP) plasmids, were first identified as a distinct family of *S. aureus* conjugative plasmids in the 1980s by the Grubb laboratory (124–127), but the complete sequence of pWBG4 (Fig. 3) was only reported in 2016 (128). The first example of this family, pWBG14, was isolated in Western Australia in 1968 and conferred resistance to MLS antibiotics (*ermA*) and spectinomycin (*spc*) and production of a diffusible orange pigment. Related plasmids were subsequently identified carrying resistance to aminoglycosides and trimethoprim (*dfrD*), and more recently several pWBG4-related plasmids were implicated in the conjugative transfer of linezolid and phenicol resistance (*cfr* and *fexA*) (128, 129) in America, China, and Germany (128, 129). pWBG4 family plasmids carry an ~18-kb conjugation-gene cluster *detA-detV* which is distinct from that of pSK41/pGO1 family plasmids and uniquely includes a MobC-like conjugative relaxase (DetB) and T4CP (DetA) related to those of the mobilizable *Enterobacter cloacae* plasmid CloDF13 (128, 129). pWBG4 plasmids also encode a PriCT_1-domain replication-initiation protein common to enterococcal Inc18 plasmids (35). pWBG4 family plasmids have been isolated from both *S. aureus* and *S. epidermidis*, and transfer between and within these species has been demonstrated (125).

The pWBG749 family was also only recently sequenced and identified as being a distinct family of *S. aureus* conjugative plasmids. pWBG749 (Fig. 3) was originally isolated in 1995 (29, 125, 130, 131) from colonizing *S. aureus* present in remote indigenous communities in Western Australia, but more recent sequencing has identified variants isolated as early as 1985 (36, 128, 132–135). pWBG749 encodes a RepA_N initiation protein, possesses a type IB partitioning system (29), and carries an ~23-kb conjugation gene cluster *smpA-X* (136) (Fig. 3). While most identified pWBG749 family plasmids lack antimicrobial-resistance determinants, members carrying resistance to penicillin (*blaZ*), aminoglycosides (*aacA-aphD*), and vancomycin (*vanA*) have been isolated (130–133, 137). Despite their frequent cryptic nature, pWBG749 family plasmids likely have an extensive impact on antimicrobial resistance dissemination through their so-far unique ability to horizontally mobilize large multiresistance plasmids in addition to smaller plasmids (131).

The mechanism by which pWBG749 mobilizes nonconjugative plasmids was only recently recognized as a naturally occurring phenomenon, potentially explaining why it was previously overlooked (128, 136). Most characterized plasmid mobilization systems described require a mobilizable plasmid to carry its own relaxase gene (138). Plasmids capable of being mobilized by pWBG749 need only carry a short "mimic" of the pWBG749 origin-of-transfer sequence (136). The conjugative relaxase of pWBG749, SmpP, along with an *oriT* specificity factor, SmpO, acts *in trans* on the *oriT* to pilot it to the mating apparatus. Approximately 50% of unique nonconjugative staphylococcal plasmids carry a pWBG749 family *oriT* mimic (136). Most common mimics are near-identical to those on pWBG749 or the closely related conjugative plasmid pWBG745 and its vancomycin resistance-encoding derivative pBRZ01 (Fig. 3) (137). Bioinformatic inspection of 397 sequenced clinical *S. aureus* isolates from the MRSA Orange County initiative of the Broad Institute (broadinstitute.org) identified just 24 contigs matching pWBG749 or pWBG745, but astonishingly, 736 *oriT* mimics were identified among the sequence contigs. This abundance is likely explained by the presence of up to three *oriT* mimic variants per plasmid on common large multidrug resis-

tance plasmids such as pIB485, pMW2, and pUSA300-HOU-MR (Fig. 2) and on small RCR plasmids (128, 136, 138).

Analogous to pWBG749-like *oriT* mimics, multiple mimic types that resemble the pSK41 *oriT* sequence were also detected recently on numerous staphylococcal plasmids (139). Although pSK41 could not mobilize the variants tested, it would seem likely that other pSK41 family plasmids encoding relaxation systems with appropriate specificity might also mediate relaxase-*in trans* mobilization. Recent bioinformatic analyses have revealed that nearly all theta replicating *S. aureus* plasmids carry at least one *oriT* mimic sequence, with many possessing both pWBG749 and pSK41 types, suggesting that the capacity for conjugative mobilization via the relaxase *in trans* mechanism is widespread (138).

TRANSPOSABLE ELEMENTS

Insertion sequences and composite transposons detected in staphylococci are listed in Table 1, and unit transposons are presented in Table 2. For many, designation as a transposable element is based on possession of diagnostic characteristics, such as terminal inverted repeats, flanking insertion sequences and/or target duplications, presence of an open reading frame encoding a transposase homolog, and/or detection in varied genetic contexts, rather than formal demonstration of mobility in a *recA* recombination-defective host. As outlined for specific elements below, in addition to facilitating the translocation of genes between replicons, insertion sequence elements are increasingly being shown to play more subtle roles in phenotypic expression and genome evolution.

Inverted copies of IS*256* flank the composite transposon Tn*4001* (Figs. 2 and 3; Table 1) (82). Tn*4001* and elements related to it are responsible for the emergence of linked resistance to the aminoglycosides, gentamicin, tobramycin, and kanamycin in staphylococci, having been found on the chromosome and theta replicating plasmids (Figs. 2 and 3) in clinical *S. aureus* and coagulase-negative staphylococcal isolates from around the world (6, 25). Indeed, some of the earliest reported gentamicin-resistant strains, dating from 1975, have been shown to carry a Tn*4001*-like structure on the chromosome (140). Resistance is mediated by the gene *aacA-aphD*, which encodes a bifunctional enzyme possessing both aminoglycoside acetyltransferase and phosphotransferase activities (75). Transposons closely related to Tn*4001* are also evident in enterococci (141) and streptococci (142). In enterococci, IS*256* and related insertion sequences are also associated with elements encoding resistance to vancomycin (143) and erythromycin (144). IS*256* also appears to be contributing to the spread of the *cfr* multiresistance gene, forming a composite-like structure with a copy of the related enterococcal element IS*Enfa4* (145, 146).

Roles in gene expression and regulation have been attributed to IS*256* via both transcriptional promotion and insertional inactivation. Transcription of the *aacA-aphD* resistance gene of Tn*4001* is directed by a complete promoter located within the end of the upstream copy of IS*256*, and it has also been found that a small flanking deletion can result in a −35-like sequence at the terminus of that element forming part of a hybrid promoter which mediates a higher level of aminoglycoside resistance (147). Additionally, roles for IS*256* hybrid promoters have been demonstrated in the expression of methicillin resistance (148, 149). Insertional inactivation of genes by IS*256* has been shown to modulate expression of several phenotypes, including virulence and glycopeptide resistance. Alternating IS*256* insertions and excisions in the *ica* operon responsible for the synthesis of the polysaccharide intercellular adhesin mediate phase-variable biofilm formation in *S. epidermidis* strains responsible for indwelling device infections (150). Notably, biofilm phase variation has also been attributed to IS*256* insertions that affect global regulatory circuits, including the *sarA* global regulator, the quorum-sensing *agr* system, and *rsbU* which positively regulates the σ^B stress response factor (151, 152). IS*256* inactivation of the *tcaA* locus was found to increase the teicoplanin resistance of a glycopeptide intermediate-resistant *S. aureus* strain (153), and IS*256* insertion into the promoter region of the *walKR* two-component regulator system similarly resulted in decreased glycopeptide susceptibility (154, 155). Insertion into the promoter repressor of toxins gene, *rot*, was also found to enhance the virulence of a USA500 strain (156).

IS*256* utilizes a "copy-out-paste-in" transposition mechanism via an excised circular double-stranded DNA intermediate (157–159). Its transposase contains an N-terminal helix-turn-helix domain that mediates binding to the element's terminal inverted repeats (160). The capacity of IS*256* to mediate phase variability has been linked to a capacity for precise excision of the element and the target duplication generated during insertion (150). Interestingly, this excision behavior occurs independently of the element's transposase and is instead thought to involve host-mediated illegitimate recombination acting on the flanking target duplications (161).

IS*257* (also known as IS*431*) has been found in diverse genetic contexts in staphylococci, variously asso-

TABLE 1 Insertion sequences and composite transposons[a]

IS[b]	Tn[c]	Associated resistance(s)/phenotype(s)	Determinant(s)	TD[d] (bp)
IS*256*	Tn*4001*[e]	Gentamicin/kanamycin/tobramycin	*aacA-aphD*	8
		Phenicols/lincosamides/oxazolidinones/pleuromutilins/streptogramin A	*cfr*	
IS*257*[f]		Kanamycin/neomycin/paromomycin/tobramycin	*aadD*	8
		Kanamycin/neomycin	*aphA-3*	
		Bacitracin	*bcrAB*	
		Bleomycin	*ble*	
		Cadmium	*cadDX*	
		Trimethoprim	*dfrK*	
		MLS	*ermC*	
		Fosfomycin	*fosB5*	
		Fusidic acid	*fusB*	
		Mupirocin	*ileS2 (mupA)*	
		Lysostaphin immunity factor	*lif*	
		Preprolysostaphin	*lss*	
		Antiseptics/disinfectants	*qacC*	
		Streptothricin	*sat4*	
		Tetracycline	*tetK*	
		Tetracycline	*tetL*	
		Streptogramin A	*vat*(A)	
		Streptogramin A/pleuromutilins/lincosamides	*vga*(A)	
		Streptogramin B	*vgb*(A)	
	Tn*4003*	Trimethoprim	*dfrA*	
	Tn*4004*	Mercury	*merAB*	
	Tn*6072*	Gentamicin/kanamycin/tobramycin	*aacA-aphD*	
	Tn*6072*	Spectinomycin	*spc*	
IS*1181*				8
IS*1182*	Tn*5405*[g]	Streptomycin	*aadE*	8
	Tn*5405*	Kanamycin/neomycin	*aphA-3*	
	Tn*5405*	Streptothricin	*sat4*	
IS*1272*	Tn*Sha1*	Triclosan	*fabI*	Absent
	Tn*Sha2*	Triclosan	*fabI*	
IS*1293*		Lysostaphin immunity factor	*lif*	Unknown
		Preprolysostaphin	*lss*	
IS*21-558*[f]		Phenicols/lincosamides/oxazolidinones/pleuromutilins/streptogramin A	*cfr*	6
		Lincosamides	*lsa*(B)	
IS*Enfa4*		Phenicols/lincosamides/oxazolidinones/pleuromutilins/streptogramin A	*cfr*	8
IS*Sau10*		Kanamycin/neomycin/paromomycin/tobramycin	*aadD*	8
		Trimethoprim	*dfrK*	
		MLS	*ermC*	
		MLS	*ermT*	
		Tetracycline	*tetL*	
IS*Sha1*				8

[a]See Paulsen et al. (25), Firth and Skurray (26), Fu et al. (237), Allignet and El Solh (238), Chen et al. (239), Kadlec and Schwarz (240), Highlander et al. (232), O'Neill and Chopra (241), de Vries et al. (242), and the text for references. Additional elements detected in genome sequences but not described here (e.g., IS*Sau1* through IS*Sau8* and IS*Sau11*) are listed in ISfinder (http://www-is.biotoul.fr).

[b]In the case of IS, association is based on probable involvement in the acquisition and dissemination of the resistance.

[c]Associated composite transposons are shown.

[d]TD, target duplication.

[e]Tn*3851* and Tn*4031* are likely to be similar or identical to Tn*4001*.

[f]IS*257* is also known as IS*431*, and IS*21-558* is also known as IS*Sau9*.

[g]Tn*3854* may be similar to Tn*5405*.

ciated with a number of determinants (Table 1). The prevalence of resistance genes flanked by copies of this element resulted in the designation of several such composite structures as transposons, *viz.*, Tn*4003* and Tn*4004*. Although the possibility that these structures can behave as traditional composite transposons cannot be excluded, such an organization is probably a consequence of the transposition mechanism of IS*257*. This insertion sequence element is thought to undergo nonresolved replicative transposition (26, 103). The expected outcome of such an event involving two replicons is cointegration, so that the replicons become fused with a directly repeated copy of IS*257* at each junction, as has been experimentally demonstrated by Needham et al. (162). The IS*257*-like element IS*Sau10* is also found in such configurations (163, 164). Many IS*257*-

TABLE 2 Unit transposons[a]

Transposon	Family	Associated resistance(s)	Determinant(s)	TD[b] (bp)
Tn*551*	Tn*3*	MLS	*ermB*	5
Tn*1546*		Vancomycin/teicoplanin	*vanHAX*	5
Tn*552*[c]	Tn*7*	Penicillins	*blaZ*	6/7
Tn*5404*		Streptomycin	*aadE*	6
		Kanamycin/neomycin	*aphA-3*	
		Streptothricin	*sat4*	
Tn*554*[d]	Tn*554*	MLS	*ermA*	Absent
		Spectinomycin	*spc*	
ψTn*554*		Cadmium	*cadA*	Absent
Tn*558*		Chloramphenicol/florfenicol	*fexA*	Absent
Tn*559*		Trimethoprim	*dfrK*	Absent
Tn*5406*		Streptogramin A/pleuromutilins/lincosamides	*vga*(A)*v*	Absent
Tn*6133*		MLS	*ermA*	Absent
		Spectinomycin	*spc*	
		Streptogramin A/pleuromutilins/lincosamides	*vga*(E)	

[a]See Paulsen et al. (25), Firth and Skurray (26), and the text for references.
[b]TD, target duplication.
[c]Tn*3852*, Tn*4002*, and Tn*4201* are likely to be similar or identical to Tn*552*.
[d]Tn*3853* is likely to be similar or identical to Tn*554*.

flanked segments represent plasmids cointegrated into larger plasmids (Figs. 2 and 3) or the chromosome (26, 103). Frequently observed deletion events adjacent to IS*257* may result from intramolecular transposition of IS*257*. However, replicon fusions and sequence deletions have also been shown to have resulted from homologous recombination between pre-existing copies of IS*257* (43).

IS*257* is a member of the IS*6* family of insertion sequences (159). Another member of this family, IS*26*, which is found flanking resistance genes with increasing prevalence in Gram-negative bacteria, has recently been shown to undergo a second "conservative" mode of transpositional movement (165, 166); the process was IS*26* transposase dependent and *recA* independent in *Escherichia coli*. It involves the insertion of a single copy of the element and a flanking DNA fragment (often containing a resistance gene), termed a translocatable unit (TU), adjacent to an existing copy of IS*26* in another replicon. The resulting composite structure can be equivalent to an IS*26*-mediated cointegration event into an "IS*26*-less" replicon, but importantly, movement of an IS*26* TU was found to occur at a much higher frequency than cointegrate formation mediated by transposition of just an IS*26* copy. As a consequence, the presence of IS*26* in a replicon predisposes it to the acquisition of IS*26* TUs. The capacity of IS*257* to promote movement via comparable TUs is yet to be investigated, but the similar properties of these elements in their distinct hosts, particularly their prevalence in gene arrays punctuated by directly repeated insertion sequence copies, suggests it is a distinct possibility (165, 166).

The activities of IS*257* are illustrated by the pSK41 family plasmids (Fig. 3). It would seem that IS*257*-mediated cointegration has enabled these conjugative plasmids to collect functions, in the form of smaller plasmids, as they move horizontally through bacterial populations. Insertions and flanking deletions mediated by this element provide a mechanism for the inactivation or removal of deleterious sequences, such as redundant replication function.

Interestingly, short (148-149 bp) elements, named ISLE39 and ISLE49, that lack any transposase coding sequence but possess terminal inverted repeats similar to those of IS*257*, have been identified in several staphylococcal species (167). These presumably represent non-autonomous miniature inverted repeat transposable elements (MITEs) that could be rendered mobile by transposase encoded by IS*257* copies present in the same cell.

A central role for IS*257* has been suggested in the evolution of staphylococcal trimethoprim resistance, including the probable capture of the resistance gene, *dfrA*, from the chromosome of an *S. epidermidis* strain (26, 103). Furthermore, an IS*257*-hybrid promoter is responsible for transcription of this determinant (103, 168). In some plasmids, the high-level trimethoprim resistance typically conferred by this determinant has been moderated as a consequence of IS*257*-associated flanking deletions. An analogous IS*257*-hybrid promoter

transcribes the *tetA*(K) tetracycline resistance gene of a cointegrated copy of pT181 within type III SCC*mec* elements (see below), and several other resistance genes are likely to be similarly promoted (102, 169).

One or more copies of IS*257* are carried by most types of staphylococcal cassette chromosome *mec* (SCC*mec*) elements (where it is commonly denoted IS*431*; see below). As well as flanking various resistance determinants within these elements, in several *SCCmec* types an IS*257* copy has mediated deletion of the *mecI* and part of the *mecR1* regulatory genes, resulting in derepressed transcription of the *mecA* methicillin resistance gene (170). Equivalent *mecI-mecR1* deletions are associated with another insertion sequence in some other SCC*mec* types, which instead contain a remnant of IS*1272*, ψIS*1272* (see below) (171–173). Multiple intact copies of IS*1272* have been found on the chromosomes of *S. aureus* and coagulase-negative staphylococcal strains, and it is particularly prevalent in *Staphylococcus haemolyticus* (4, 172, 174). IS*1272* is also associated with the elements Tn*Sha1* and Tn*Sha2*, which encode the *fabI* determinant responsible for resistance to the biocide triclosan (175); these elements are believed to target palindromic sequences and not generate target duplications during transposition.

IS*21-558* (also called IS*Sau9*) and derivatives thereof have been linked with the *cfr* multiresistance gene in a range of contexts and hosts (145). Its name reflects the fact that it is a member of the IS*21* family of insertion sequence elements and that it was initially detected within a variant of Tn*558* (176), which itself carries a *fexA* determinant that mediates chloramphenicol and florfenicol efflux (177). Within the Tn*558* variant, two directly repeated IS*21-558* copies formed a composite configuration flanking both *cfr* and the *lsa*(B) lincosamide resistance gene (176).

In addition to the high-level glycopeptide resistance VanA element Tn*1546* of enterococcal origin (27) described above, one other Tn*3*-type transposon, Tn*551*, has been detected in staphylococci. This MLS (*ermB*) transposon is closely related to Tn*917* from *E. faecalis* but confers constitutive rather than inducible MLS resistance (7). Tn*551* was originally identified on the β-lactamase/heavy-metal resistance plasmid, pI258 (Fig. 2) but has subsequently been shown to transpose to numerous sites in other large plasmids and the chromosome. Despite a tendency to "hot-spot," it has served as a valuable tool for mapping and mutagenesis studies (7, 178).

Tn*552* and Tn*5404* are Tn*7* family unit transposons. Tn*552* appears to be restricted to a very limited set of insertion sites on the chromosome and in plasmids (25). In theta replicating plasmids (Figs. 2 and 3), the insertion sequence sites of Tn*552* and Tn*552*-like elements are normally located within resolution sites upstream of resolvase (recombinase) genes encoded by the plasmids (43, 86, 87, 179). An invertible segment can be formed between resolution sites that are present in both the plasmid and transposon (180). In some ICE*6013* elements (see below) Tn*552* is also found proximal to the probable recombinase genes (181). This property is shared with Tn*5035*-like elements from Gram-negative bacteria, which are termed "*res* site hunters" (182). Despite such insertional specificity, Tn*552*-type transposons are thought to represent the source of all staphylococcal β-lactamase genes (76). However, in many plasmids, only remnants of such transposons remain, presumably as a consequence of multiple insertion events and/or rearrangements mediated by the recombinase systems present on both the transposon and plasmids.

Tn*5404* is thought to have resulted from the transposition of a chromosomal element to a plasmid in a clinical *S. aureus* strain (183). This transposon shares an invertible segment (between resolution sites) with an adjacent copy of Tn*552*, such that an entire copy of one or the other of these elements is generated depending on the orientation of the segment (183). Another invertible segment within Tn*5404* represents a composite structure designated Tn*5405* (183), which encodes resistance to aminoglycosides (*aadE*, *aphA*) and streptothricin (*sat4*) (184). Tn*5405* is bounded by copies of IS*1182*, an element related to IS*1272* (see above) (185). Moreover, one of the IS*1182* elements flanking Tn*5405* is interrupted by a copy of IS*1181*, which is both prevalent and active in *S. aureus* (1, 185, 186).

An incomplete vestige of an element related to IS*1181* has been detected on the plasmid pACK1 from *Staphylococcus simulans* biovar *staphylolyticus* ATCC1362. This element, designated IS*1293*, is located at one end of a segment that contains the genes encoding preprolysostaphin (*lss*) and lysostaphin immunity factor (*lif*) (187). It is likely that the *lss-lif* segment of this plasmid, which possesses a truncated remnant of IS*257* at its other end, has been acquired through horizontal transfer (187). Numerous copies of another IS*1181*-like element, IS*Sha1*, have been found in *S. haemolyticus* strains (188).

The MLS- and spectinomycin-resistance element Tn*554* is unusual in that it lacks terminal inverted repeats and generates no target sequence duplications upon insertion (189). It inserts at an efficiency approach-

ing 100% into a unique site in the *S. aureus* and *S. epidermidis* chromosomes, termed *att554* (189), within the *radC* gene (190), with a preference for one orientation. Secondary insertions can be generated in the chromosome or plasmids, albeit at much lower frequency, if *att554* is absent or occupied by a pre-existing copy of Tn*554*. However, natural isolates have been identified that carry this or related elements, such as ψTn*554* encoding cadmium resistance, at different secondary sites, including some located in SCC*mec* regions (see below) (1, 179). Insertion of Tn*554* is dependent on two tyrosine-based site-specific recombinases encoded by the *tnpA* and *tnpB* genes and the product of a third, *tnpC*, that influences efficiency and orientation specificity (191, 192). The recombinases of Tn*554* are related to integrases of bacteriophage and ICEs such as Tn*916* (see below). The Tn*554*-like elements, Tn*558* (177), Tn*559* (190), Tn*5406* (193), and Tn*6133* (194) exhibit equivalent insertion site specificity but encode additional or alternative resistance determinants.

INTEGRATIVE AND CONJUGATIVE ELEMENTS

ICEs are mobile genetic elements that share characteristics of both conjugative plasmids and chromosomally integrating elements such as transposons and prophage. ICEs primarily exist within the host chromosome but can excise, circularize, and transfer via conjugation. ICEs encode all genes necessary for mating-pair formation and DNA transfer. The broad impact of ICEs on an organism's capacity for horizontal gene transfer and dissemination of antimicrobial resistance and virulence factors is significant. However, perhaps due to the often-changing classification of ICEs as genomic islands, transposons, or conjugative transposons and the evolving differences in naming conventions (195, 196), the roles of ICEs in *S. aureus* gene transfer are not yet as widely recognized as those of their plasmid and bacteriophage counterparts.

Identified *S. aureus* ICEs can be grouped into two families: those identical or closely related to the Tn*916* ICE and those related to the distinct ICE*6013* family (16). The conjugation gene clusters of Tn*916* and ICE*6013* family elements both carry conjugation genes related to those of *Bacillus subtilis* ICE*Bs1* (Fig. 4) and more distantly, plasmids such as *Clostridium perfringens* pCW3 and *Enterococcus* spp. pIP501 (197). *S. aureus* Tn*916* and ICE*6013* family elements vary in their integration mechanism, insertion site preference, and antimicrobial-resistance gene cargo.

Tn*916* elements are extremely promiscuous in Gram-positive organisms, and those identified in *S. aureus* are often identical to those in other Gram-positive genera. Tn*916* transfer from *E. faecalis* to *S. aureus* and transfer between *S. aureus* strains has been experimentally demonstrated (15, 198). The 18-kb Tn*916* element carries genes for conjugation, integration, excision, and the tetracycline-minocycline-resistance gene *tetM*. Tn*916* integration is mediated by the tyrosine recombinase Int, and excision is stimulated by Int and the excisionase Xis. Int preferentially targets pairs of poly-A and poly-T tracts separated by a 6-bp spacer for Tn*916* insertion (199). Tn*916* excision is stimulated in the presence of tetracycline and other ribosome-targeting antibiotics through an antiattenuation mechanism resulting in the derepression of *xis*; therefore, antibiotic exposure directly drives the spread of Tn*916*-mediated resistance (200–202).

Various distinct Tn*916*-like elements have been described in *S. aureus*, but the most well-documented example is the Tn*5801* subfamily. Tn*5801* (Fig. 4) was originally identified in *S. aureus* strain Mu50 (1) and carries conjugation and resistance genes (*tetM*) closely related to those of Tn*916* in both sequence and organization. Tn*5801* is larger (25 kb) than Tn*916* and harbors several Tn*5801*-specific genes within the conjugation-gene cluster. *int* and *xis* genes of Tn*5801* are distinct from those of Tn*916*, as is the integration preference for the 3′ end of the conserved GMP-synthase gene, *guaA*. Tn*5801* transfer between *S. aureus* strains has also been demonstrated (15). Like Tn*916* elements, Tn*5801* elements have been identified in human and animal-associated *S. aureus* and are particularly prevalent in *S. aureus* clonal complex CC398 (15, 203, 204). Both elements are also abundant in other animal-associated *Staphylococcus* spp. (204, 205), suggesting that agricultural and companion animals may be reservoirs of Tn*916* and Tn*5801*-carrying staphylococci.

ICE*6013* is the most recently identified family of *S. aureus* ICEs, and although it is also related to Tn*916* and Tn*5801*, it is more closely related to *B. subtillis* ICE*Bs1* than any other *S. aureus* element (206) (Fig. 4). Unlike Tn*916* and Tn*5801*, ICE*6013* family members generally lack antimicrobial-resistance determinants, although the first discovered ICE*6013* element in *S. aureus* HDG2 carries a Tn*552* insertion conferring penicillin resistance, and the insertion has immobilized this copy of ICE*6013* (181, 206) (Fig. 4). As well as being one of the smallest known families of ICE, with a minimum size of 12.7 kb, ICE*6013* is the only known ICE to utilize a DDE family transposase for its integration and excision and has an insertion preference for the sequence

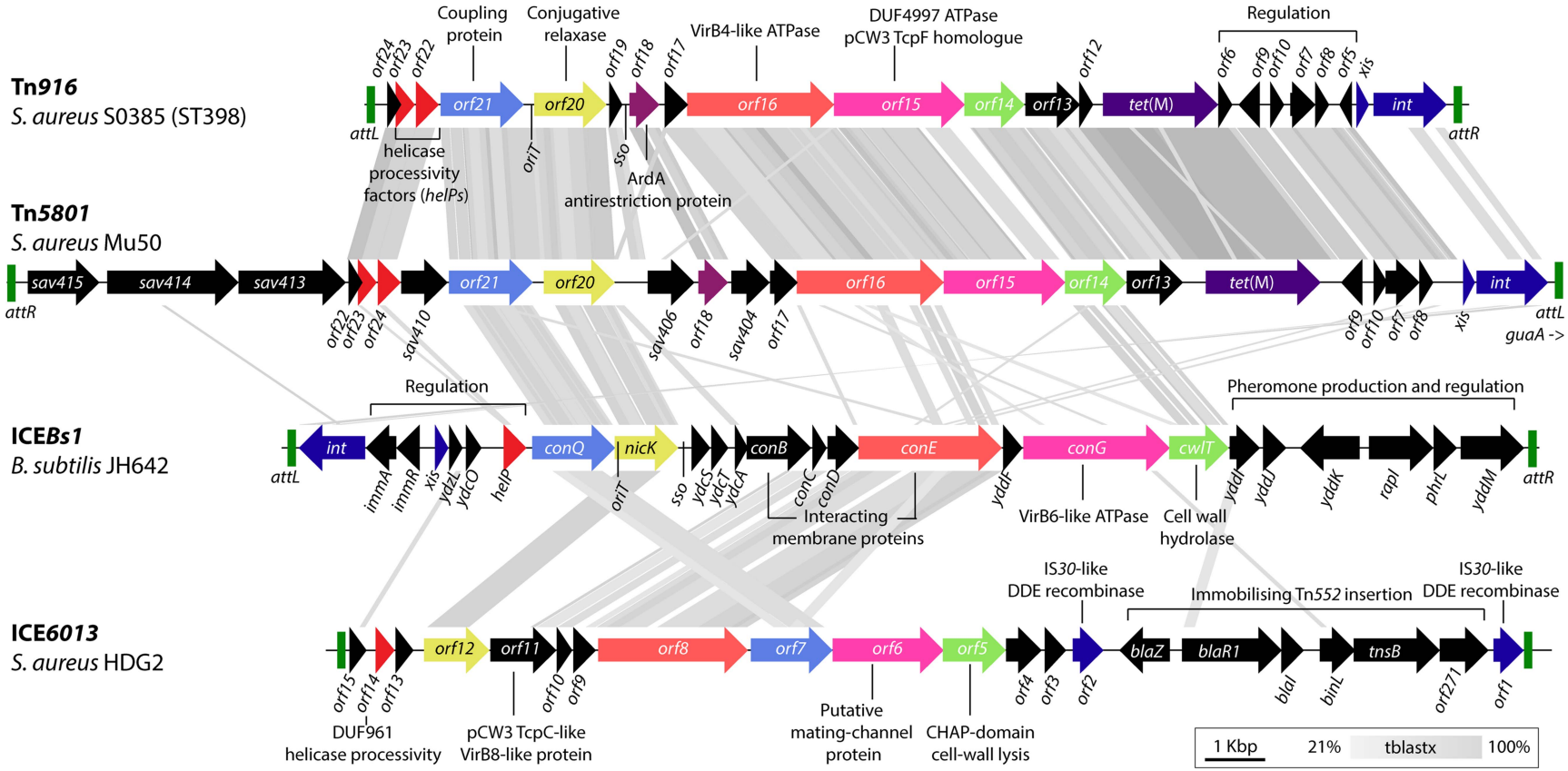
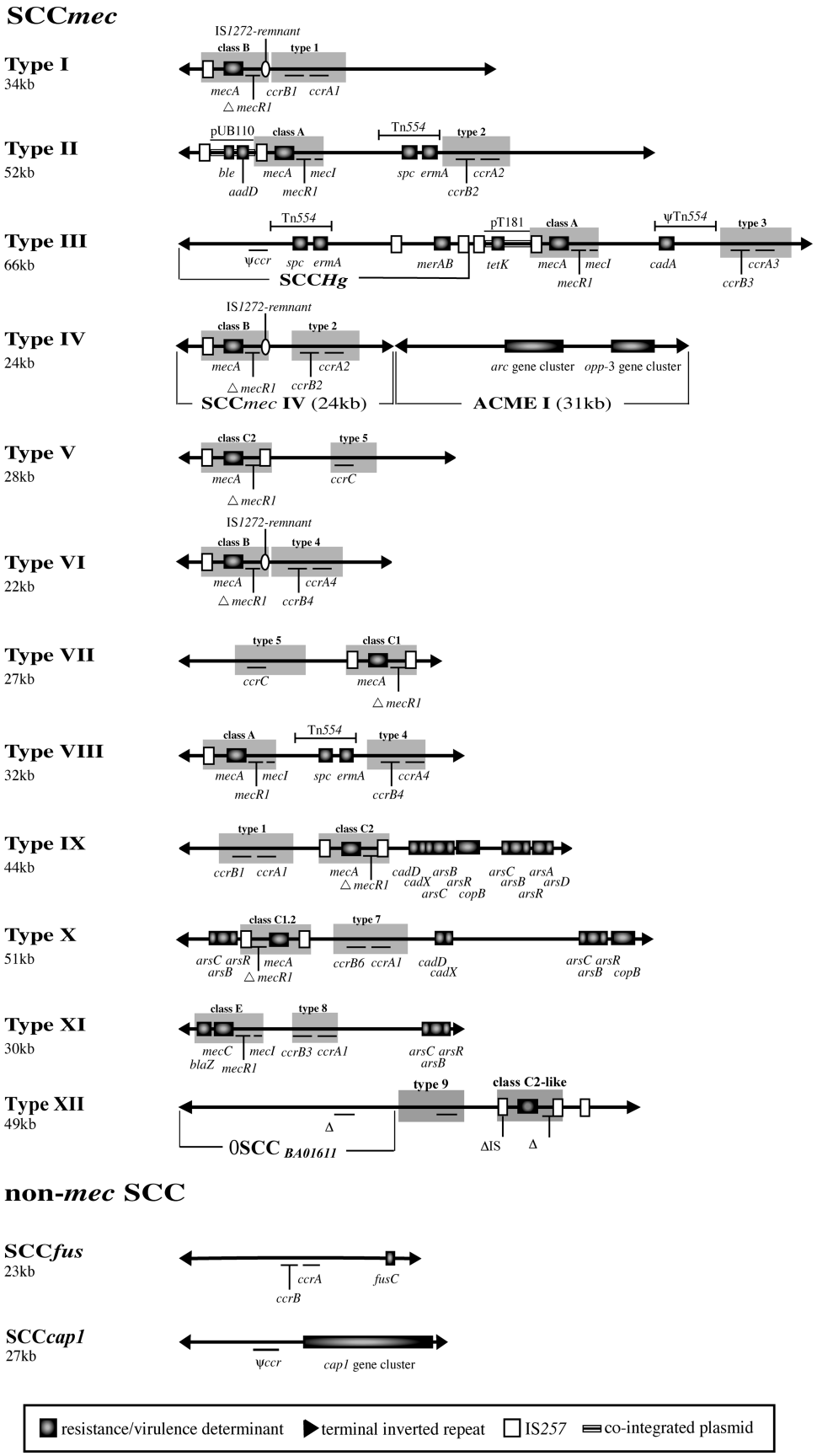


FIGURE 4 Genetic organization of Tn*916*, Tn*5801*, ICE*Bs1*, and ICE*6013*. A comparison of Tn*916*, Tn*5801*, ICE*Bs1*, and ICE*6013* constructed using EasyFig (233). Amino acid similarity comparisons (20% lower cutoff) using tblastx and are shown as gray shading between regions on each ICE. Genes with likely common functions—regardless of sequence similarity—are similarly colored. Positions of the conjugative origin of transfer (*oriT*) and single-strand origin of replication (*sso*) are indicated if known. Recombinase attachment/target sites are indicated by green rectangles. Annotations were collated from previously published figures for each ICE (15, 181, 197, 204, 206, 234, 235).


SCCmec
Type I
34kb
IS1272-remnant
class B
type 1
mecA
ccrB1
ccrA1
△mecR1
Type II
52kb
pUB110
class A
Tn554
type 2
ble
mecA
mecI
spc
ermA
ccrA2
aadD
mecR1
ccrB2
Type III
66kb
Tn554
pT181
class A
ψTn554
type 3
ψccr
spc
ermA
merAB
tetK
mecA
mecI
cadA
ccrA3
mecR1
ccrB3
SCCHg
Type IV
24kb
IS1272-remnant
class B
type 2
mecA
ccrA2
arc gene cluster
opp-3 gene cluster
△mecR1
ccrB2
SCCmec IV (24kb)
ACME I (31kb)
Type V
28kb
class C2
type 5
mecA
ccrC
△mecR1
Type VI
22kb
IS1272-remnant
class B
type 4
mecA
ccrA4
△mecR1
ccrB4
Type VII
27kb
type 5
class C1
ccrC
mecA
△mecR1
Type VIII
32kb
class A
Tn554
type 4
mecA
mecI
spc
ermA
ccrA4
mecR1
ccrB4
Type IX
44kb
type 1
class C2
ccrB1
ccrA1
mecA
△mecR1
cadD
cadX
arsB
arsR
arsC
copB
arsC
arsA
arsB
arsD
arsR
Type X
51kb
class C1.2
type 7
arsC
arsR
arsB
mecA
△mecR1
ccrB6
ccrA1
cadD
cadX
arsC
arsR
arsB
copB
Type XI
30kb
class E
type 8
mecC
mecI
ccrB3
ccrA1
arsC
arsR
arsB
blaZ
mecR1
Type XII
49kb
type 9
class C2-like
Δ
ΔIS
Δ
0SCC BA01611
non-mec SCC
SCCfus
23kb
ccrA
fusC
ccrB
SCCcap1
27kb
ψccr
cap1 gene cluster
resistance/virulence determinant
terminal inverted repeat
IS257
co-integrated plasmid

5′-TTTTTANNNTAAAAA-3′ (181). ICE*6013* was originally identified in the ST239 *S. aureus* strain HDG2, and related elements have been identified in 44% of distinct *S. aureus* sequence types, including the dominant American community-associated lineage USA300. Conjugation frequencies of ICE*6013* approach rates similar to that of the conjugative plasmid pSK41/pG01. Some strains harbor various combinations of up to four unique ICE*6013*, Tn*916*, and Tn*5801* family ICEs simultaneously (23, 181). The Tn*916*/Tn*5801 orf18* gene encodes the ArdA antirestriction protein (Fig. 4), which inhibits type I restriction modification systems ubiquitous in *S. aureus*, so capture of Tn*916*/Tn*5801* ICE may increase the propensity for horizontal acquisition of additional mobile elements (207–209).

In addition to their own conjugative transfer, ICEs can mobilize the transfer of compatible nonconjugative plasmids or other integrative elements (16, 210). ICE*Bs1* is able to mobilize the transfer of RCR plasmids encoding RepU replication initiators through an interaction dependent on RepU and the ICE*Bs1* T4CP, ConQ (58, 197). ICE*6013*, Tn*916*, and Tn*5801*-family ICEs share closely related ConQ homologs (Fig. 4), so it is plausible that *S. aureus* ICEs may facilitate mobilization of RCR plasmids. Indeed, ICE*Bs1*- and Tn*916*-mediated mobilization of *S. aureus* antimicrobial-resistance plasmids pC194 and pUB110 has already been demonstrated, albeit in a *B. subtilis* host (58, 211). The impact of *S. aureus* ICEs on the capacity to facilitate horizontal gene transfer of other mobile genetic elements warrants further investigation (16, 138).

STAPHYLOCOCCAL CASSETTE CHROMOSOME

Most methicillin-resistant *S. aureus* isolates contain a 20- to 70-kb DNA segment, generically termed the *mec* region, that is not present in methicillin-sensitive strains. These segments are a type of genomic island (i.e., are bounded by terminal inverted repeats and subject to horizontal transfer) called staphylococcal cassette chromosome *mec* (SCC*mec*) (179). Methicillin resistance is mediated, at least in part, by the *mecA* gene or the more recently identified *mecC* gene (212, 213), both of which encode a related low-affinity penicillin-binding protein, termed PBP2a. Two regulatory loci, *mecI* and *mecR1*, are commonly found upstream of, and transcribed divergently to, *mecA*/*mecC* (however, see discussion of IS*1272*/IS*257*, above). In combination, the *mecA*/*mecC*, *mecI*, and *mecR1* genes and associated insertion sequence(s) are referred to as the *mec* gene complex, of which several classes have been defined (A, B, C1, C2, D, and E) on the basis of structural diversity (http://www.sccmec.org).

Additionally, SCC*mec* contains the *ccr* gene complex, which comprises either a combination of *ccrA* and *ccrB* genes (one of each) or a single *ccrC* gene; different allotypes for these genes also exist, and several types of the *ccr* gene complex have been defined (http://www.sccmec.org) (214). Each *ccr* gene encodes a recombinase that mediates the excision and circularization of the cassette and its site- and orientation-specific insertion into the $attB_{SCC}$ site of the *S. aureus* chromosome and homologous sites in other staphylococcal species (179). Recently, a conserved gene located upstream of the *ccr* gene(s) has been shown to encode a DNA helicase (215) referred to as *cch* or *cch2*, and the latter is also preceded by a putative primase (*polA*). This implies that SCC elements might be able to replicate (postexcision), and multiple copies would likely facilitate the horizontal transfer process. Moreover, a gene located downstream of the *ccr* gene(s) encodes a uracil-DNA glycosylase inhibitor (SAUGI). *S. aureus* pathogenicity islands, which are specifically induced to excise and be packaged by invading bacteriophage, carry homologs of *cch*, and regulate their excision in response to phage-induced changes in uracil metabolism. These commonalities suggest that phage may also have a role in stimulating and/or facilitating SCC*mec* transfer (128, 215).

At present, SCC*mec* has been classified into 12 allotypes based on the organization of the *mec* and *ccr* gene complexes (Fig. 5) (216); subtypes also exist and are

FIGURE 5 Maps of representative SCC elements (4, 216); see text for additional references. Resistance/virulence genes, transposons, insertion sequences, and cointegrated plasmids are shown: *arsBC*, arsenic resistance; *cadA*, cadmium resistance; *cap1* genes, capsular polysaccharide (220); *fusC* (previously known as *far*), fusidic acid resistance (236); *mecA*/*mecC*, β-lactam resistance. Refer to Table 1 for the antimicrobial resistance(s) conferred by other resistance determinants. Cassette recombinase genes (*ccrA*, *ccrB*, and *ccrC*), *mecA*/*mecC* regulatory genes (*mecI* and *mecR1*), and an arginine catabolic mobile element (ACME I [227]) are also shown; *mec* classes and *ccr* types are denoted by gray shading. Note that *cch* genes, *polA*, and SAUGI are not shown and that IS*257* is also known as IS*431*.

defined by the structure of the three joining (J) regions (previously called junkyard regions) located between these gene complexes and the chromosomal regions flanking SCC*mec*. The J regions also act as a chromosomal hot-spot for the insertion of additional antimicrobial resistance determinants, often in association with transposable elements (173). These include Tn*554* or related elements encoding resistance to erythromycin and spectinomycin, or cadmium, and IS*257*-flanked segments conferring resistance to mercury, tetracycline, and/or aminoglycosides and bleomycin; the latter two segments are known to be cointegrated copies of the plasmids pT181 and pUB110, respectively (Fig. 5) (26, 103). Based on this classification system, an online tool, SCC*mec*Finder, has recently been developed for rapid whole-genome sequence-based SCC*mec* typing (217).

It is important to note that SCC*mec* elements are not exclusive to *S. aureus* and are frequently carried by coagulase-negative staphylococci. Moreover, the ubiquitous carriage of a *mecA* homolog by *Staphylococcus sciuri* has led to the suggestion that this or closely related species represent the origin of the *mec* determinants found in other species (218). In this regard, a recent study by Rolo et al. (219) indicates that *Staphylococcus vitulinus* and *Staphylococcus fleurettii* contributed to the evolution of the *mec* complex and that *S. sciuri* likely provided the mobile element into which this complex was later incorporated. In any case, it is clear that coagulase-negative staphylococci act as a reservoir from which methicillin-sensitive *S. aureus* can acquire SCC*mec*, contributing to the emergence of new methicillin-resistant *S. aureus* clones.

However, not all SCC elements encode resistance to methicillin, because related structures have also been identified (179, 220, 221). These include SCC*cap1*, which encodes a type I capsular polysaccharide (220), and SCC*fur* (also known as SCC_{476}) and SCC*Hg* (also known as SCC*mercury*), which encode resistance to fusidic acid and mercuric chloride, respectively (4, 222) (Fig. 5). Additionally, the arginine catabolic mobile element, which is broadly associated with certain coagulase-negative staphylococci (223–225), has also been identified in association with SCC*mec* allotypes, largely type IV (226). This element is a virulence/colonization determinant that utilizes SCC*mec*-encoded cassette recombinases and integrates into the $attB_{SCC}$ site (223, 227, 228) (Fig. 5). Arginine catabolic mobile element allotypes are defined by the presence or absence of the *opp*-3 and *arc* gene clusters, the latter of which encode a complete arginine deiminase pathway, and carriage of this element has been shown to enhance fitness and skin colonization (228–230).

PERSPECTIVES

Mobile genetic elements and the mechanisms of genetic exchange they facilitate/exploit act in concert to catalyze rapid microbial adaptation by providing access to an extended reservoir of niche-adaptive functions. Clinical staphylococci represent a salient illustration of the evolutionary potential this affords. Genomic technologies have greatly expanded our understanding of variability between strains (clinical and nonclinical) and the relationships between them and revealed a growing repertoire of mobile genetic elements, thereby illustrating the significance of horizontal genetic exchange. There is little doubt that the ongoing acceleration of genomics will yield further insights in the future.

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