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Association of different mobile elements to generate novel integrative elements

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Abstract. Among the more important problems in modern hospitals is the prevalence of bacterial pathogens expressing resistance to multiple antimicrobial agents. The frequency of multiresistance suggests mechanisms by which bacterial species can concentrate and efficiently exchange a variety of resistance determinants. Mechanisms by which this occurs include insertion of transposons within transposons, coalescence through the activity of insertion sequences and the employment of integrons. In some instances, more than one of these mechanisms is involved in creating large multiresistance genetic elements. The association of the elements with transferable elements or transposons may promote rapid dissemination among clinical strains, and create further opportunities for inclusion of additional resistance determinants.

Key words. Transposon; insertion sequence; integron; multiresistance.

Introduction

A frequent assumption in modeling the emergence and spread of antimicrobial resistance in bacteria is that resistance determinants confer a cost to the microorganism. This cost is perceived as reasonable in an environment in which survival is threatened by an antibiotic, but soon becomes burdensome when the threat is removed. Consequently, it is generally assumed that absent an immediate threat, resistance determinants will be lost over time. However, the modern hospital environment and in many cases the community are rich in recurrent lethal antimicrobial threats. It is therefore reasonable to assume that a microorganism's long-term interest would be served by maintaining certain resistance determinants even in the absence of an immediate need for them. Perhaps more accurately, it is conceivable that bacterial lineages retaining banks of resistance determinants will more readily establish and maintain footholds in antimicrobial-rich environments than those that do not. The question then becomes not whether there is advantage to be gained by maintaining multiple resistance determinants, but rather how best to maintain and transmit these determinants while conferring minimal costs to the microorganisms harboring them.

Two features important for establishing such bacterial lineages are stability and transmissibility of resistance determinants. Stability is necessary to avoid unnecessary loss of resistance in the absence of selective pressure and is best accomplished by integration into the bacterial chromosome. Transmissibility, on the other hand, maximizes the chance that disparate beneficial determinants will find a common host and is most commonly facilitated by incorporation into transferable plasmids. Since plasmids are by definition extrachromosomal, they are inherently less stable within a microorganism than the chromosome (though many encode segregation genes that minimize the chance that they will be lost with replication). So maximum stability and transmissibility are in a sense mutually exclusive. Perhaps not surprisingly, bacteria have resolved this issue by arriving at compromise solutions in which transferable elements are integrated into the bacterial chromosome. Under these circumstances, some stability is lost in comparison with a permanently integrated element, and some transmissibility is lost compared with a highly transmissible plasmid, but the net benefit to the microorganism is arguably greater than either of the other two situations.

Efficient replication and transfer both favor concentration of different determinants within a small area of the bacterial genome, necessitating mechanisms whereby desirable determinants may be concentrated. Several such concentration mechanisms have been described, which I will

summarize as follows: (i) transposons within transposons; (ii) coalescence through the use of insertion sequence (IS) elements and (iii) employment of integrons. These categories are by no means fixed or exclusive, and it is likely that bacteria use some combination of these mechanisms (and perhaps several yet to be described) to accomplish their aims.

It is worth noting at this point that the bacterial genome appears to be a fluid entity. Comparison of the genome of *Escherichia coli* O157-H7 with that of the previously sequenced genome of *E. coli* K-12 reveals an additional 856 kb of DNA [1]. Recent data emanating from a comparative study of 36 *Staphylococcus aureus* genomes indicate that 22% of the genome is dispensable, with many of the variable regions constituting presumed pathogenicity islands and regions of antimicrobial resistance [2]. Bacteria have evolved mechanisms over the years to address many different environmental challenges. The present description of categories of resistance gene concentration represents merely one example of tools used for many different aspects of molecular evolution.

Transposons within transposons

Tn*5253*

Incorporation of transposons within transposons is an efficient method for stockpiling resistance genes and possibly enhancing their characteristics. For example, if the target transposon has conjugative functions but the arriving transposon does not, then the resistance determinants present on the arriving transposon subsequently become conjugative with movement of the target element. Alternatively, insertion of a conjugal element into a nonconjugal element could conceivably confer conjugal abilities on the target transposon. At the very least, this strategy serves to bring resistance determinants within close proximity, potentially promoting cotransfer by techniques (such as transformation or transduction, in the absence of intrinsic conjugation capabilities) that do not directly involve functions encoded by either element.

One of the best-studied examples of a transposon within a transposon is the *Streptococcus pneumoniae* transposon Tn*5253* (originally designated *Ω cat tet*) [3]. Tn*5253* is a 65.5-kb element conferring resistance to tetracycline [via a *tet*(M) gene] and chloramphenicol [via a chloramphenicol acetyltransferase (*cat*) gene] originally found integrated into the chromosome of *S. pneumoniae* BM6001. Tn*5253* was found to be transferable between pneumococcal strains. In addition, *tet*(M) was transferable independent of *cat*, but *cat* transferred only with *tet*(M). Structural analysis of Tn*5253* revealed that it was a composite of a 47.5-kb conjugative element (encoding *cat* and designated Tn*5252*) and an inserted 18-kb conjugative transposon (designated Tn*5251*) indistinguishable from prototypical conjugative transposon Tn*916* [3, 4]. Transfer of *tet*(M) from within this composite occurred at rates of 10^{-6}–10^{-5} per recipient CFU, whereas transfer of both *cat* and *tet*(M) occurred at rates of 10^{-7}/recipient CFU. Transfer of *tet*(M) alone was association with transfer of Tn*5251* and insertion into recipient chromosomes was relatively nonspecific, whereas insertion of Tn*5253* was site-specific [5]. After excision of Tn*5251* from Tn*5253* (yielding Tn*5252*), transfer frequencies for the *cat* determinant were similar to those for Tn*5253*. Tn*5252* has now been extensively studied and found to contain, in addition to Tn*5251* and *cat*, regions responsible for transfer, site-specific integration and excision, ultraviolet (UV) resistance and cytosine methylation [5–8].

SCC*mec*

Recent data suggest that the methicillin resistance regions of clinical *Staphylococcus aureus* (MRSA) isolates, though not demonstrably transferable, exhibit 'transposon-within-transposon' features. Methicillin resistance is conferred by PBP2a, a low-affinity penicillin-binding protein that is encoded by the *mecA* gene, located within the SCC*mec* (staphylococcal chromosomal cassette) region of the MRSA chromosome [9, 10]. *mecA* is not native to *S. aureus*, and may have been acquired from coagulase-negative staphylococcal species (a similar gene, capable of conferring resistance to methicillin resistance when unregulated, is intrinsic to *Staphylococcus sciuri*) [11]. The *mecA* gene is frequently under the regulatory control of upstream genes *mecI* and *mecR1*, although its expression may also be regulated by coresident *β*-lactamase regulatory genes [12]. Nosocomial MRSA strains are frequently resistant to several unrelated classes of antibiotics, including erythromycin and chloramphenicol [13]. It has been known for several years that these other resistance phenotypes are frequently the result of integration of either an erythromycin resistance transposon (Tn*554*) or a chloramphenicol resistance plasmid (pUB110) in the vicinity of *mecA*. In fact, since the SCC*mec* region has never been shown to be transferable, investigators have used the relationships between Tn*554* and *pbp2a* to define specific lineages of MRSA strains [14].

Substantial insight into the structure of SCC*mec* regions across different MRSA strains has recently been provided through the sequencing efforts of Keichi Hiramatsu and colleagues. After defining the complete nucleotide sequence of an MRSA SCC*mec* region in 1999, these investigators followed up with a detailed comparison of SCC*mec* regions of the original sequenced isolate and two additional MRSA strains [9, 15]. The results are summarized in figure 1. In essence, there is a basic SCC*mec* region that is composed of the *mecI-mecR1-mecA* resistance determinant, some component of its regulatory region and

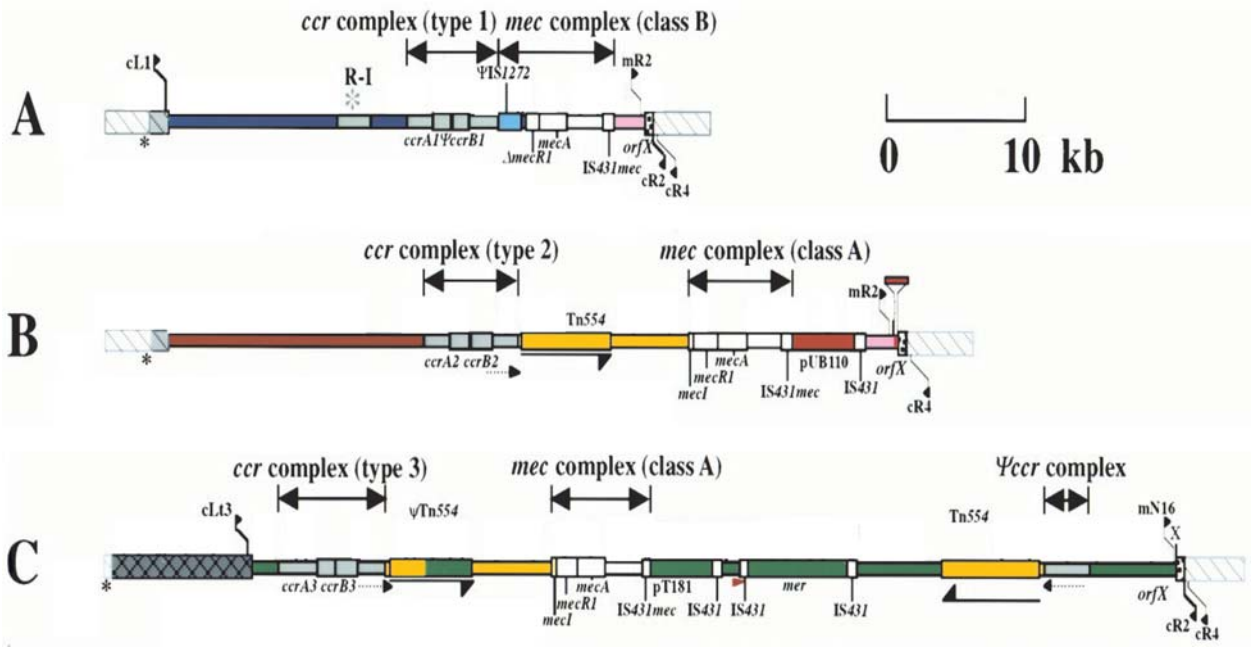

Figure 1. Comparison of three different SCC*mec* regions from strains of *Staphylococcus aureus*. Regions in white represent the highly conserved *mec* regions of the three elements. Regions in grey represent the three different *ccr* complexes (see text for details). Regions in dark blue are unique to *ccr* complex (type 1) SCC*mec*. Regions in red are unique to *ccr* complex (type 2) SCC*mec*. Regions in green are unique to *ccr* complex (type 3) SCC*mec*. Regions in yellow are common to *ccr* complex (types 2 and 3) SCC*mec*s. Individual genes and IS elements are indicated below the maps. Limits of the *ccr* and *mec* regions, and limits of individual transposons or putative transposons, are indicated above the map. Adapted from [15] with permission from the publisher.

putative transposition genes *ccrA* and *ccrB*. The sequences of the *mecI-mecR1-mecA* genes in the three isolates are highly conserved (>99% identity) [15]. The sequences of the *ccrA* and *ccrB* genes are somewhat less conserved (resulting in alleles designated 1–3), but are highly homologous. Chromosomal sequences flanking the right end of the SCC*mec* insertion are highly conserved in all three strains, whereas the sequences flanking the left end are varied. The remainder of SCC*mec* is varied in the three different classes, in many cases reflecting the insertion of various transposons. In particular, Tn*554* is inserted into both type II and type III SCC*mec*s, although in different locations relative to the *mecA* gene. Type III also has a variant of Tn*554* (*Ψ*Tn*554*) that confers cadmium resistance rather than erythromycin and spectinomycin resistance found upstream of *mecA*. Both type II and type III have small, mobilizable staphylococcal plasmids integrated immediately downstream of *mecA*. In type II, this integrated plasmid is pUB110, conferring chloramphenicol resistance; in type III, it is pT181, conferring tetracycline resistance [15]. In both instances, the integrated plasmids are flanked by staphylococcal IS element IS*431*, and in the type III SCC*mec* pT181 is linked to a mercury resistance operon. Type I SCC*mec* contains no resistance determinants other than *mecA*, but does contain sequences that are unique to it (in blue in fig. 1) relative to the other two sequences.

The entire SCC*mec* region can excise from the staphylococcal chromosome through the actions of the gene products encoded by *ccrA* and *ccrB*, so it is presumed to be a mobile element [10]. However, it has never been conclusively shown to be transferable in vitro to other staphylococcal strains, so it does not appear to be a conjugative mobile element. One is left with the impression, after analyzing these different SCC*mec* elements, of a primordial SCC*mec* element containing only *ccrA*, *ccrB* and the *mecI*, *mecR1* and *mecA* genes, or perhaps containing the *ccrA* and *ccrB* genes alone. Over time and under selective pressures (including antibiotic pressure), the region has diverged by accepting different transposons, plasmids and other integrating elements to create the multiresistance MRSA strains now so common in hospitalized patients. More recently, MRSA strains have emerged in patients from the community in the US [16]. These strains are unlike the nosocomial strains in that they are susceptible to most other classes of antimicrobial agents. It will be of considerable interest to analyze the SCC*mec* regions of these new strains, to determine whether they have an even more spare form of the presumed vestigial SCC*mec* element.

Tn*vamp*

We have recently identified a transposon within *Enterococcus faecium* that encodes VanB-type glycopeptide re-

sistance. This 33-kb element, designated Tn*5382*, possesses several open reading frames homologous to ORFs found within Tn*916*-like conjugative transposons [17]. Consistent with the similarity to Tn*916*, we have demonstrated that Tn*5382* can circularize, forming a joint between the ends of the element in its circular form that is similar to joints identified in conjugative transposons. In *E. faecium* strain C68 (and several others), Tn*5382* is integrated into the *E. faecium* chromosome immediately downstream of the *pbp5* gene, which confers high levels of resistance to ampicillin [17]. Transfer of vancomycin resistance from C68 to *E. faecium* recipient strains is universally associated with cotransfer of PBP5-mediated ampicillin resistance in a larger mobile element at least 120 kb in size [18]. We have tentatively designated this element Tn*vamp*. The precise mechanism of transfer and the structure of the larger element have yet to be characterized, but the transfer of the two determinants together is highly reminiscent of other transposon-within-a-transposon structures.

Coalescence through the use of IS elements

IS elements, small (generally less than 2.5 kb) segments of DNA encoding nothing other than their own mobility, are frequent components of multiresistance elements in gram-positive bacteria. The simplest transposon type that incorporates IS elements is the composite transposon, in which the ends of mobile elements are formed by similar or identical IS elements, and these elements encode the functions that result in the mobility of the larger element [19]. The simplest and most widespread composite transposon in gram-positive bacteria is probably the 4.7-kb Tn*4001*, consisting of the *aac-6′-aph2″* bifunctional aminoglycoside-modifying-enzyme gene flanked by inverted copies of IS*256* [20]. Tn*4001* and similar elements have been identified in a wide range of Gram-positive species. A larger composite transposon found in *Enterococcus faecalis* is Tn*1547*, a 65-kb element in which the VanB glycopeptide resistance operon is flanked by copies of IS elements similar to IS*256* [21]. Although the VanA glycopeptide resistance operon is universally associated with a Tn*3*-family transposon designated Tn*1546*, in some instances it has achieved mobility through the action of flanking copies of IS*1216* [22]. Perhaps the best-studied example of IS elements converging to create a large composite element is Tn*5385* [23].

Tn*5385*

The structure of Tn*5385* is shown in figure 2. Tn*5385* was first described in strains of *E. faecalis* isolated during an outbreak of β-lactamase-producing enterococci in 1986 [24, 25]. Tn*5385* is an ~65 kb element whose ends are directly repeated copies of the insertion sequence IS*1216* (IS*1216* is found frequently in enterococci, often integrated within or near Tn*1546*) [26]. The left-end IS*1216* of Tn*5385* flanks an OFR with 97% identity to an ORF from plasmid pSM19035 from *Streptococcus pyogenes*, suggesting a potential origin for this region [23]. To the right of this ORF is located the *aadE* gene, encoding high-level resistance to streptomycin (Sm in fig. 2). To the right of the *aadE* gene is Tn*5381*, a Tn*916*-like conjugative transposon [27]. To the right of Tn*5381* in Tn*5385* lies a third copy of IS*1216*, which is flanked by sequences 72% identical to the relaxase gene of small, mobilizable staphylococcal plasmid pS194 (Mob in fig. 2). This arrangement suggests that this copy of IS*1216* may have mediated a cointegration between streptococcal and staphylococcal plasmids [28]. To the right of the staphylococcal sequences lies a structure indistinguishable from Tn*4001* [20]. The right end of Tn*4001* within Tn*5385* interrupts sequences identical to the replication region (Rep in fig. 1) of broad host-range enterococcal plasmid pAMβ1 [29], which itself has been interrupted by a deletion derivative of erythromycin resistance transposon Tn*917*. This replication region is likely nonfunctional, since the region known to be the origin of pAMβ1 replication has been deleted by the insertion of IS*256* [29]. To the right of the replication region gene *repC* lie directly repeated copies of staphylococcal IS element IS*257* (identical to IS*431* – see above) flanking a mercury resistance operon (Mer in fig. 2, similar if not identical to the *mer* region of *SCCmec* – see above). To the right of the *mer* region lie sequences identical to those found on staphylococcal β-lactamase plasmids, including a version of the β-lactamase transposon Tn*552* [30] (Bla in fig. 2) whose regulatory gene *blaR1* has been inserted into by a copy of IS*256* [31].

Tn*5385* actually represents both the transposon-within-transposon and the coalescence of IS elements models. There are five individual transposons or potential transposons within Tn*5385*. Two are known to be functional. Tn*5381* behaves in a fashion similar to Tn*5251* (see above discussion of Tn*5253*) in that it transfers either independently or with Tn*5385*. Tn*5384* is a composite element (limits delineated above the map in fig. 2) whose ends are formed by the left IS*256* of the Tn*4001*-like element within Tn*5385* and the directly repeated downstream IS*256* inserted into the *blaR1* gene upstream of the β-lactamase gene *blaZ*. This transposon does not appear to be conjugative, but can transpose into transferable plasmids, resulting in transconjugants expressing resistance to gentamicin, erythromycin and mercury [32]. The Tn*917*-like element within which erythromycin resistance is encoded is no longer functional, since the transposition genes have been deleted [33]. However, the presence of characteristic direct repeats flanking this insertion suggests that it was a

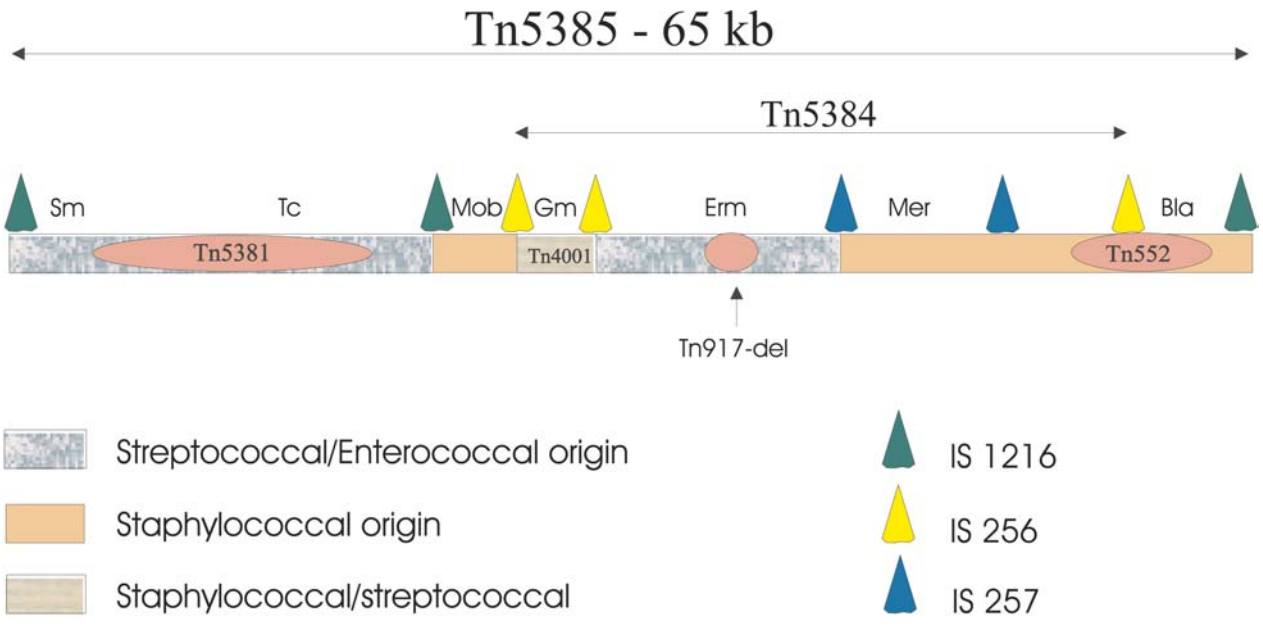

Figure 2. Graphic depiction of the structure of Tn*5385* (see text for discussion and references). Legend indicates the presumed origins (based on previously reported sequence data) of the different regions of the mobile element. The figures marking the placement of insertion sequences are also indicated in the legend. Transposons within the larger element are indicated in various ways, depending on their degree of overlap and so on Resistance genes and other important loci are noted above the element. Bla-β-lactamase gene; Erm-erythromycin-resistance determinant; Gm, *aac6′-aph2″* bifunctional aminoglycoside resistance gene; Mer, mercuric chloride resistance determinant; Mob, mobilization region similar to that found on small staphylococcal plasmids; Sm, *aadE* streptomycin resistance gene; Tc, *tet*(M) tetracycline-minocycline resistance determinant within Tn*5381*. Tn*917*-del signifies the region that contains a version of Tn*917* in which the transposition genes have been deleted. Reprinted from [47] with permission from the publishers.

functional transposon when it arrived. Structurally, the region surrounding the β-lactamase determinant is identical to those described in staphylococcal transposon Tn*552*, although actual transposition of these elements has been very difficult to demonstrate [30]. Finally, the IS*257*/*431* elements flanking the mercury resistance determinant imply that it is transposable, since these elements have been shown to be transposable, but its actual mobility has never been demonstrated.

It is not possible to conclusively reconstruct the events that led to the formation of Tn*5385*. However, its structure suggests possible evolutions. The primary elements are most likely pAMβ1 (the broad host range plasmid) and IS*1216*. It is conceivable that an IS*1216*-containing variant of pAMβ1 traveled from an *Enterococcus* to *S. pyogenes* and underwent IS*1216*-mediated cointegration with an *aadE*-, Tn*5381*-containing streptococcal plasmid. This composite plasmid could then have transferred into *S. aureus*, where IS*1216*-mediated cointegration of the pAMβ1-based plasmid with a Tn*4001*-containing small, mobilizable plasmid occurred. If this larger plasmid then cointegrated with a staphylococcal β-lactamase plasmid, followed by deletion of extraneous segments (the selective advantage conferred by the resistance genes presumably led to their surviving these different 'downsizings') of the larger plasmid through the action of internal IS elements, something approximating the final version of Tn*5385* could result. Conjugative transfer back to *E. faecalis* followed by IS*1216*-mediated integration into the chromosome and ultimately deletion of the plasmid replication mechanism (favoring maintenance in the chromosome) would lead to the final form of Tn*5385* [23]. This scenario obviously represents only one of many pathways that could result in the final version of Tn*5385*.

SXT^{MO10}

An apparent combination of the transposon-within-a-transposon and IS element strategy has recently been described in *Vibrio cholerae* O139. Waldor and colleagues [34] had previously described the SXT element (closely related to the IncJ element R391 from *Providencia rettgeri*) [35], an ~100-kb conjugative element that integrates site specifically into the *E. coli* chromosome at the 5′ end of the *prfC* gene. Since this element is conjugative, self-transmissible and integrating, these investigators refer to it as a constin. Excision of the SXT element to form a nonreplicating circular intermediate and its subsequent insertion into the *E. coli* chromosome are mediated by an element-encoded integrase [36].

The SXT element from *V. cholerae* MO10 (SXT^{MO10}) encodes multiple phenotypes, including resistance to

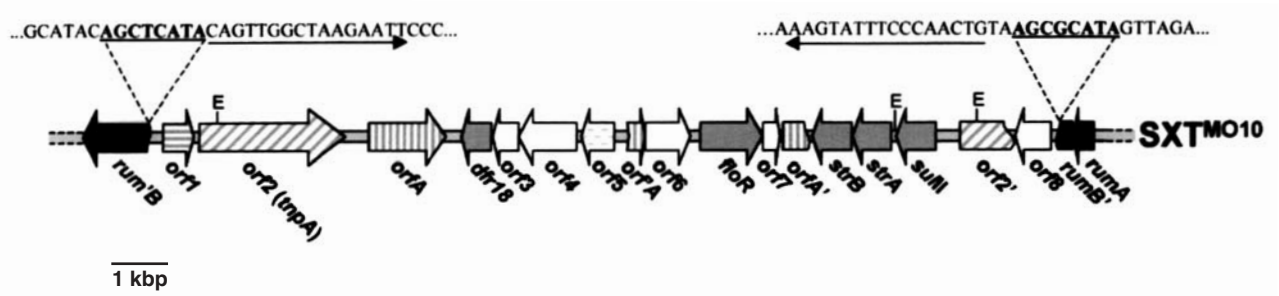

Figure 3. Organization of the region containing antibiotic resistance genes in SXT^{MO10}. The positions of the different ORFs are indicated below the map. *dfr18* designates gene conferring resistance to trimethoprim. *floR* designates gene encoding resistance to chloramphenicol and florfenicol. *strA* and *strB* designate genes conferring resistance to streptomycin. *sulII* designates genes responsible for resistance to sulfamethoxazole. Other putative ORF functions are described in the text. Adapted from [37] with the permission of the publisher.

trimethoprim, chloramphenicol, streptomycin and sulfamethoxazole [37]. Detailed structural analysis of SXT^{MO10} indicates that genes encoding these four resistance phenotypes are all clustered within a circumscribed 9.4-kb region of the element that is part of a larger (17.2 kb) element. The structure of the 17.2-kb region, shown in figure 3, appears to have inserted into the SXT element, interrupting the *rumAB* operon. Consistent with this region arriving by transposition is the identification of 8-bp direct repeats flanking a 16-bp imperfect inverted repeat at the point of *rumB* interruption [37]. In addition, at the left end of the element are found ORFs (*orfs* 1 and 2 in fig. 3) with homology to previously described transposases. A second *orf*, *orfA*, is present in complete or incomplete forms at three points within the element, flanking several of the resistance genes, suggesting a possible role in its assembly and arrangement. *orfA* exhibits similarity to the C terminus of a transposase found in *Pseudomonas pseudoalcaligenes*. These authors also reported SXT-like element-containing *V. cholerae* strains that did not express as many resistance phenotypes as MO10, and provided compelling circumstantial evidence that these less-resistant SXT variants could have evolved from SXT^{MO10} by recombinational events leading to deletion of parts of the 17.2-kb segment [37]. It is of interest that the arrangement of these resistance genes is in some respects reminiscent of the structure of integrons (see below), but the specific sequences found characteristically within integrons do not appear to be present in these strains.

IS element-mediated affects on structure and function

Attempts to follow lineages of bacterial strains often rely on interpretations of maps resulting from restriction enzyme digestions of regions within which resistance determinants rest. Original interpretations of the regions within which the VanA glycopeptide resistance operon was located were complicated by the fact that the almost universally present transposon (Tn*1546*) was frequently deleted or altered by the presence of a variety of IS elements [26]. Only careful hybridization studies and extensive sequencing have allowed us to associate VanA with Tn*1546* in virtually all instances, although in many cases only remnants of the original transposon remain.

IS elements can also have an impact on the expression of resistance genes within mobile elements. Analysis of a large number of *mecA* regions in *S. aureus* and coagulase-negative staphylococci indicates that expression of methicillin resistance is related to the presence of the upstream regulatory sequences *mecI* and *mecR1* [38]. Higher level and constitutive expression of methicillin resistance is facilitated by deletion of these upstream regulatory sequences. Katayama and colleagues have demonstrated that loss of these regulator genes in *S. aureus* or *Staphylococcus haemolyticus* is associated with insertion of either IS*1272* or IS*431* in *mecR1* gene (see figs 1, 4), resulting in deletion of *mecI* and portions of *mecR1* [38]. In addition to the regulatory changes associated with these IS*431* insertions, the presence of directly repeated copies of this mobile element flanking the *mec* region creates the potential for a new transposable element.

Employment of integrons

As ubiquitous as IS elements and transposons appear to be in the development of composite elements in Gram-positive bacteria, integrons serve a similar function in many Gram-negative species. More than 40 antimicrobial resistance genes have been described within integrons [39]. Integrons are frequently located within transposons or plasmids, enhancing their mobility. In essence, integrons serve as ‘sinks’ for important resistance genes. The typical integron is characterized by the presence of an integrase gene that acts on two substrates. The *attI* site is the site of attachment for new resistance cassettes within the integron. The 59-base element (or *attC*) is the substrate within the circularized cassette gene. The reaction catalyzed by the integrase gene results in incorporation of the circular-

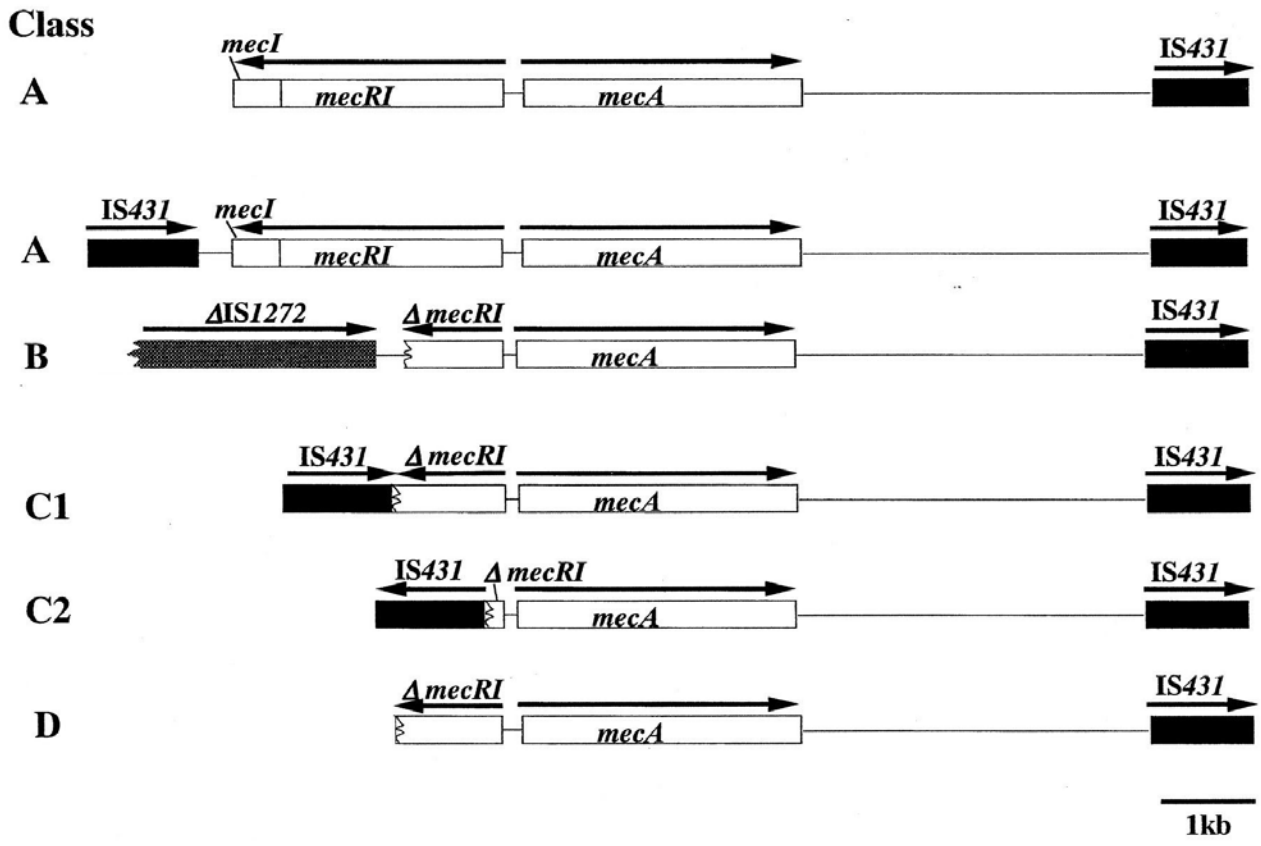

Figure 4. Genetic organization of the *mecA* gene complex in staphylococci. The structures of five classes of *mec* complexes found in MRSA and MRC-NS are shown. In all five classes, IS*431R* was present downstream of *mecA*. The class A *mec* complex has the structure *mecI-mecR1-mecA*-IS*431mec*. In *S. haemolyticus*, the class A *mec* complex was accompanied by an upstream IS*431* copy (IS*431L*). The class B *mec* complex has the structure ΨIS*1272-ΔmecR1-mecA*-IS*431*. Class C1 and C2 *mec* complexes have a deletion in the left side of the class A *mec* complex associated with an insertion of IS*431*. In class C1, IS*431L* is inserted in the same orientation as *mecA*, and in class C2, IS*431L* is inserted in the opposite orientation relative to *mecA*. Class D has a deletion without any insertion sequence adjacent to the deletion point. Arrows above the genes indicate the direction of transcription. Adapted from [38] with the permission of the publisher.

ized cassette gene within the integron (fig. 5) [40]. Expression of the integrated gene is driven by a promoter located within the integrase, with the intensity of expression being related to the nature of the promoter and the distance of the gene from the promoter (genes further down the line are expressed less well) [41]. Although there are no documented examples as yet of integrons within conjugative transposons, there are examples of these structures located within conjugative plasmids. They have also been identified within nonconjugative transposons or defective transposons [42, 43].

Although integrons have most often been described in association with antibiotic resistance genes, it appears likely that they are actually ancient elements present in many different bacteria and associated with the acquisition of genes that become defining characteristics for various genera or species within genera. Recent work performed in *V. cholerae* has identified structures designated 'super-integrons' [44]. The originally described super-integron spanned 126 kb and contained at least 179 cassettes. Integrases associated with these super-integrons are similar to those present in the more well-known varieties, and are able to facilitate integration of cassettes in the same manner [45]. None of these vestigial elements has been shown to be mobile, but they may have served as the origin of the mobile integrons now so prevalent in clinical bacterial isolates. In fact, one-fifth of the described integron antimicrobial resistance cassettes have *attC* sites identical to those described on super-integrons [45]. Known antimicrobial resistance cassettes have not yet been identified within these super-integrons, yet several possible progenitor cassette candidates (exhibiting some homology to known resistance genes) have been identified. A more detailed understanding of the prevalence and functions of these super-integrons will greatly inform our understanding of bacterial genetic evolution.

Conclusions

Our scientific understanding of the mechanisms by which bacteria move and concentrate important genetic

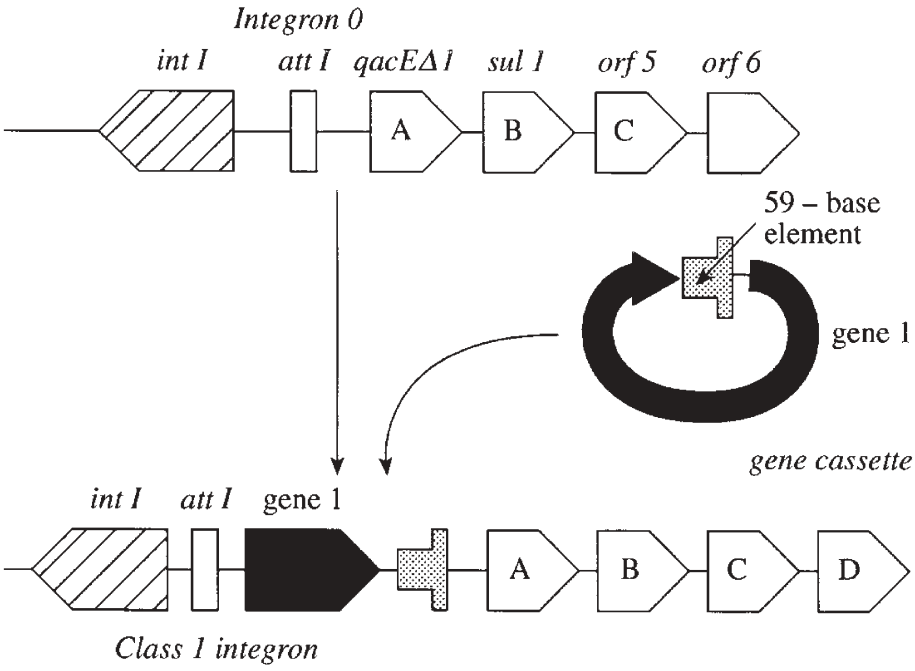

Figure 5. Structure of a class 1 integron. The basic structure is shown in the upper aspect of the figure. The integrase gene (*intI*) is located upstream of the *attI* site. Downstream of *attI* lie open reading frames conferring resistance to quarternary ammonium compounds (*qacE1*) and sulfonamides (*sulI*) in addition to two open reading frames (*orfs* 5 and 6) of unknown function. Incoming cassettes are circularized open reading frames that integrate into the integron by an integrase catalyzed interaction between *attI* and the 59-base element (*attC*). The final structure has the integrated cassette present upstream of the 50-base element and the other genes and transcribed from a promoter located within the *intI*. Figure reprinted from [40] with permission of the publishers

determinants has of necessity focused on the movement of antimicrobial resistance determinants. It is important to understand, however, that this emphasis on resistance determinants reflects bias introduced both by the clinical importance of resistance (to us) and the relative ease with which the movement of resistance can be followed in the laboratory. It is easy to comprehend that IS elements are by nature independent of resistance genes, and associated with them only by chance and the application of selective pressure. More subtle is the observation that more than 70% of the unique restriction sites in Tn*916* are found within the 2-kb region of the tetracycline resistance gene, suggesting that this transposon evolved as a more general purpose broad host-range element [46], and that the arrival of the resistance determinant occurred very late in its evolution. The observation that super-integrons are ancient elements involved in overall evolution and differentiation of bacterial genera and species further underscores the more correct notion that mechanisms of mobility and concentration of genes in bacteria are well developed, intrinsic characteristics of many different bacterial species. That these mechanisms have been employed by human pathogens to answer the challenge of widespread antimicrobial usage simply reflects a diversion of resources to address a current need, rather than a creative endeavor that requires considerable effort. With this growing knowledge, it is hoped that our repeated underestimation of these formidable opponents' abilities will be a thing of the past.

1 Hayashi T., Makino K., Ohnishi M., Kurokawa K., Ishii K., Yokoyama K. et al. (2001) Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. DNA Res. **8:** 11–22
2 Fitzgerald J. R., Sturdevant D. E., Mackie S. M., Gill S. R. and Musser J. M. (2001) Evolutionary genomics of *Staphylococcus aureus*: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. Proc. Natl. Acad. Sci. USA **98:** 8821–8826
3 Ayoubi P., Kilic A. O. and Vijayakumar M. N. (1991) Tn*5253*, the pneumococcal omega (cat tet) BM 6001 element, is a composite structure of two conjugative transposons, Tn*5251* and Tn*5252*. J. Bacteriol. **173:** 1617–1622
4 Provvedi R., Manganelli R. and Pozzi G. (1996) Characterization of conjugative transposon Tn*5251* of *Streptococcus pneumoniae*. FEMS Microbiol. Lett. **135:** 231–236
5 Vijayakumar M. N. and Ayalew S. (1993) Nucleotide sequence analysis of the termini and chromosomal locus involved in site-specific integration of the streptococcal conjugative transposon Tn*5252*. J. Bacteriol. **175:** 2713–2719
6 Munoz-Najar U. and Vijayakumar M. N. (1999) An operon that confers UV resistance by evoking the SOS mutagenic response in streptococcal conjugative transposon Tn*5252*. J. Bacteriol. **181:** 2782–2788
7 Sampath J. and Vijayakumar M. N. (1998) Identification of a DNA cytosine methyltransferase gene in conjugative transposon Tn*5252*. Plasmid **39:** 63–76

8 Srinivas P., Kilic A. O. and Vijayakumar M. N. (1997) Site-specific nicking in vitro at ori T by the DNA relaxase of Tn*5252*. Plasmid **37:** 42–50

9 Ito T., Katayama Y. and Hiramatsu K. (1999) Cloning and nucleotide sequence determination of the entire mec DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. Antimicrob. Agents Chemother. **43:** 1449–1458

10 Katayama Y., Ito T. and Hiramatsu K. (2000) A new class of genetic element, staphylococcus cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **44:** 1549–1555

11 Wu S. W., de Lencastre H. and Tomasz A. (2001) Recruitment of the *mecA* gene homologue of *Staphylococcus sciuri* into a resistance determinant and expression of the resistant phenotype in *Staphylococcus aureus*. J. Bacteriol. **183:** 2417–2424

12 Hackbarth C. J. and Chambers H. F. (1993) *blaI* and *blaR1* regulate β-lactamase and PBP 2a production in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **37:** 1144–1149

13 Chambers H. F. (1997) Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin. Microbiol. Rev. **10:** 781–791

14 Kreiswirth B., Kornblum J., Arbeit R. D., Eisner W., Maslow J. N., McGeer A. et al. (1993) Evidence for a clonal origin of methicillin resistance in *Staphylococcus aureus*. Science **259:** 227–230

15 Ito T., Katayama Y., Asada K., Mori N., Tsutsumimoto K., Tiensasitorn C. et al. (2001) Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **45:** 1323–1336

16 From the Centers for Disease Control and Prevention (1999) Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus* – Minnesota and North Dakota, 1997–1999. JAMA **282:** 1123–1125

17 Carias L. L., Rudin S. D., Donskey C. J. and Rice L. B. (1998) Genetic linkage and cotransfer of a novel, *vanB*-containing transposon (Tn*5382*) and a low-affinity penicillin-binding protein 5 gene in a clinical vancomycin-resistant *Enterococcus faecium* isolate. J. Bacteriol. **180:** 4426–4434

18 Hanrahan J., Hoyen C. and Rice L. B. (2000) Geographic distribution of a large mobile element that transfers ampicillin and vancomycin resistance between *Enterococcus faecium* strains. Antimicrob. Agents Chemother. **44:** 1349–1351

19 Galas D. J. and Chandler M. (1989) Bacterial insertion sequences. In: Mobile DNA, pp. 109–162, Berg D. E. and Howe M. M. (eds), American Society for Microbiology, Washington, DC

20 Lyon B. R., May J. W. and Skurray R. A. (1984) Tn*4001*: a gentamicin and kanamycin resistance transposon in *Staphylococcus aureus*. Mol. Gen. Genet. **193:** 554–556

21 Quintiliani R. Jr and Courvalin P. (1996) Characterization of Tn*1547*, a composite transposon flanked by the IS*16* and IS*256*-like elements, that confers vancomycin resistance in *Enterococcus faecium* BM4281. Gene **172:** 1–8

22 Heaton M. P., Discotto L. F., Pucci M. J. and Handwerger S. (1996) Mobilization of vancomycin resistance by transposon-mediated fusion of a VanA plasmid with an *Enterococcus faecium* sex pheromone-response plasmid. Gene **171:** 9–17

23 Rice L. B. and Carias L. L. (1998) Transfer of Tn*5385*, a composite, multiresistance element from *Enterococcus faecalis*. J. Bacteriol. **180:** 714–721

24 Rhinehart E., Smith N. E., Wennersten C., Gorss E., Freeman J., Eliopoulos G. et al. (1990) Rapid dissemination of β-lactamase-producing, aminoglycoside-resistant *Enterococcus faecalis* among patients and staff on an infant-toddler surgical ward. N. Engl. J. Med. **323:** 1814–1818

25 Rice L. B., Eliopoulos G. M., Wennersten C., Goldmann D., Jacoby G. A. and Moellering R. C. Jr (1991) Chromosomally-mediated β-lactamase production and gentamicin resistance in *Enterococcus faecalis*. Antimicrob. Agents Chemother. **35:** 272–276

26 Woodford N., Adebiyi A. M., Palepou M. F. and Cookson B. D. (1998) Diversity of VanA glycopeptide resistance elements in enterococci from humans and nonhuman sources. Antimicrob. Agents Chemother. **42:** 502–508

27 Rice L. B., Marshall S. H. and Carias L. L. (1992) Tn*5381*, a conjugative transposon identifiable as a circular form in *Enterococcus faecalis*. J. Bacteriol. **174:** 7308–7315

28 Projan S. J. and Novick R. (1988) Comparative analysis of five related staphylococcal plasmids. Plasmid **19:** 203–221

29 Swinfield T.-J., Oultram J. D., Thompson D. E., Brehm J. K. and Minton N. P. (1990) Physical characterization of the replication region of *Streptococcus faecalis* plasmid pAMβ1. Gene **87:** 79–90

30 Rowland S. J. and Dyke K. G. H. (1990) Tn*552*, a novel transposable element from *Staphylococcus aureus*. Mol. Microbiol. **4:** 961–975

31 Rice L. B. and Marshall S. H. (1994) Insertions of IS*256*-like element flanking the chromosomal β-lactamase gene of *Enterococcus faecalis* CX19. Antimicrob. Agents Chemother. **38:** 693–701

32 Rice L. B., Carias L. L. and Marshall S. H. (1995) Tn*5384*, a composite enterococcal mobile element conferring resistance to erythromycin and gentamicin whose ends are directly repeated copies of IS*256*. Antimicrob. Agents Chemother. **39:** 1147–1153

33 Bonafede M. E., Carias L. L. and Rice L. B. (1997) Enterococcal transposon Tn*5384*: evolution of a composite transposon through cointegration of enterococcal and staphylococcal plasmids. Antimicrob. Agents Chemother. **41:** 1854–1858

34 Waldor M. K., Tschape H. and Mekalanos J. J. (1996) A new type of conjugative transposon encodes resistance to sulfamethoxazole, trimethoprim, and streptomycin in *Vibrio cholerae* O139. J. Bacteriol. **178:** 4157–4165

35 Hochhut B., Beaber J. W., Woodgate R. and Waldor M. K. (2001) Formation of chromosomal tandem arrays of the SXT element and R391, two conjugative chromosomally integrating elements that share an attachment site. J. Bacteriol. **183:** 1124–1132

36 Hochhut B. and Waldor M. K. (1999) Site-specific integration of the conjugal *Vibrio cholerae* SXT element into *prfC*. Mol. Microbiol. **32:** 99–110

37 Hochhut B., Lotfi Y., Mazel D., Faruque S. M., Woodgate R. and Waldor M. K. (2001) Molecular analysis of antibiotic resistance gene clusters in *Vibrio cholerae* O139 and O1 SXT constins. Antimicrob. Agents Chemother. **45:** 2991–3000

38 Katayama Y., Ito T. and Hiramatsu K. (2001) Genetic organization of the chromosome region surrounding *mecA* in clinical staphylococcal strains: role of IS*431*-mediated *mecI* deletion in expression of resistance in *mecA*-carrying, low-level methicillin- resistant *Staphylococcus haemolyticus*. Antimicrob. Agents Chemother. **45:** 1955–1963

39 Hall R. M. (1997) Mobile gene cassettes and integrons: moving antibiotic resistance genes in gram-negative bacteria. Ciba Found Symp **207:** 192–202

40 Bennett P. M. (1999) Integrons and gene cassettes: a genetic construction kit for bacteria. J. Antimicrob. Chemother. **43:** 1–4

41 Collis C. M. and Hall R. M. (1995) Expression of antibiotic resistance genes in the integrated cassettes of integrons. Antimicrob. Agents Chemother. **39:** 155–162

42 Radstrom P., Skold O., Swedberg G., Flensburg J., Roy P. H. and Sundstrom L. (1994) Transposon Tn5090 of plasmid R751, which carries an integron, is related to Tn*7*, Mu, and the retroelements. J. Bacteriol. **176:** 3257–3268

43 Brown H. J., Stokes H. W. and Hall R. M. (1996) The integrons In0, In2, and In5 are defective transposon derivatives. J. Bacteriol. **178:** 4429–4437
44 Mazel D., Dychinco B., Webb V. A. and Davies J. (1998) A distinctive class of integron in the *Vibrio cholerae* genome. Science **280:** 605–608
45 Rowe-Magnus D. A., Guerout A. M., Ploncard P., Dychinco B., Davies J. and Mazel D. (2001) The evolutionary history of chromosomal super-integrons provides an ancestry for multiresistant integrons. Proc. Natl. Acad. Sci. USA **98:** 652–657
46 Oggioni M. R., Dowson C. G., Smith J. M., Provvedi R. and Pozzi G. (1996) The tetracycline resistance gene *tet*(M) exhibits mosaic structure. Plasmid **35:** 156–163