Chapter

The Tn*916*/Tn*1545* Family of Conjugative Transposons

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Abstract The conjugative transposon Tn*916* was first discovered in the late 1970s and is, together with the related conjugative transposon Tn*1545*, the paradigm of a large family of related conjugative transposons known as the Tn*916*/Tn*1545* family, which are found in an extremely diverse range of bacteria. With the huge increase in bacterial genomic sequence data available, due to the widespread use of next generation sequencing, more putative conjugative transposons belonging to the Tn*916*/Tn*1545* family are being reported. Many of these are capable of excision, integration and conjugation. Nearly all of the Tn*916*/Tn*1545*-like elements discovered to date encode tetracycline resistance however, increasingly resistance to other antimicrobials is being found. Some of the members of the Tn*916*/Tn*1545* family of elements are composite structures which contain smaller mobile genetic elements which are also capable of transposition. Tn*916*/Tn*1545*-like elements themselves are also found within larger and more complex elements. This review will give an overview of the current knowledge of the Tn*916*/Tn*1545* family of conjugative transposons highlighting recently characterized composite elements carrying additional and novel resistance genes.

Introduction

*The Conjugative Transposon Tn*916

The existence of chromosomally, as opposed to plasmid encoded transferable resistance was first suspected in the late 1970s. When *Enterococcus faecalis* strain DS16 was mated with the plasmid-free *E. faecalis* strain JH2–2, some transconjugants resistant to tetracycline contained the Tn*916* determinant linked to the co-resident plasmid pAD1 which had also transferred from DS16. In addition, derivatives of DS16 devoid of pAD1 were capable of transferring tetracycline resistance to recipient strains. Transconjugants (plasmid-free) from such matings could subsequently act as donors in the transfer of tetracycline resistance. Further work showed that tetracycline resistance was conferred by an integrative element which was called a conjugative transposon and was designated Tn*916*.[1] This was the first conjugative transposon found to carry an antibiotic resistance gene and it was hypothesized that it may explain the widespread presence of tetracycline resistance among streptococci at the time.[1]

The genetic organization of Tn*916,* and many other mobile genetic elements (MGEs), is modular.[2-4] The modules are involved in conjugation, excision and integration (recombination),

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Figure 1. Schematic of Tn*916* showing the four functional modules: conjugation (dark gray/blue); regulation (light gray/green); recombination (black/red) and the accessory gene *tet*(M) (gray/black with white dots). Schematic adapted from: Roberts AP, Mullany P. Trends Microbiol 2009; 17:251-8;[4] ©2009 with permission from Elsevier.

regulation and accessory functions which are not involved in mobility or regulation (Fig. 1).[4] Each of these functional modules will be considered separately and in detail below.

The Tn*916* Family of Conjugative Transposons; An Ever Expanding Family of MGEs

In the past few decades, numerous mobile genetic elements with similarities to Tn*916* have been characterized (Fig. 2). While the conjugation and the regulatory genes are generally conserved the genes encoding the excisionases, integrases or recombinases and the accessory genes vary (Fig. 2). A number of elements within the family also contain insertions of smaller MGEs encoding resistance to other antimicrobials e.g., macrolides[5,6] and mercury.[7] Group II introns and IS elements have also been found in some of the Tn*916*/Tn*1545* family of MGEs (Fig. 2).[8-10] Tn*916*-like elements are found in a wide range of bacterial species belonging to at least 36 genera spanning six phyla (Fig. 3), giving the Tn*916*/Tn*1545* family an exceptionally broad host range.[4]

Here we present an overview of the current knowledge of the biological functions of Tn*916* encoded proteins and explore their genetic diversity.

The Functions of the Transposon Encoded Proteins by Module

Recombination

All of the Tn*916*/Tn*1545* family of MGEs possess a recombination module which is located at one end of the element with the direction of transcription leading out of that end of the element (Fig. 1).[11-14] In Tn*916*, this consists of two genes: encoding a tyrosine integrase, IntTn, and an excisionase, XisTn. Excision begins with the introduction of staggered endonucleolytic cuts made at each end of the element generating single-stranded, non-complementary hexanucleotides at each end of the element termed the coupling sequences.[13,15] The coupling sequences then join forming a covalent bond creating a circular intermediate molecule with a heteroduplex at the joint, while the target site from which the element has excised is also ligated. On integration into a target site, heteroduplex regions are produced on either side of the conjugative transposon which are then resolved by DNA repair or replication. One study has shown that Tn*916* is also capable of inversion in its target site. PCRs performed on DNA extracted from a broth culture of *Enterococcus faecium* DPC3675 which carries one copy of Tn*916* showed the ends of the transposon in both orientations within the target site,[16] work in our lab has shown that this may be a general property of Tn*916*/Tn*1545*-like elements (Roberts et al., unpublished).

Tn*916* and Tn*1545* both encode a tyrosine recombinase which are highly related to each other.[14,17] There is variation however between tyrosine recombinases associated with different Tn*916*-like elements e.g., Int*6000*[18] is more related to the tyrosine integrases of staphylococcal pathogenicity islands than it is to the integrases of Tn*916* and Tn*1545*, probably reflecting different recombination events between different MGEs which have led to the formation of

Figure 2. The structure of various members of the Tn*916*/Tn*1545* family is shown. Functional modules are represented as shown in the key. The organisms from which the elements were isolated are shown to the left in brackets. Mobility is denoted by a capital M on the right. Schematic adapted from: Roberts AP, Mullany P. Trends Microbiol 2009; 17:251-8;[4] ©2009 with permission from Elsevier.

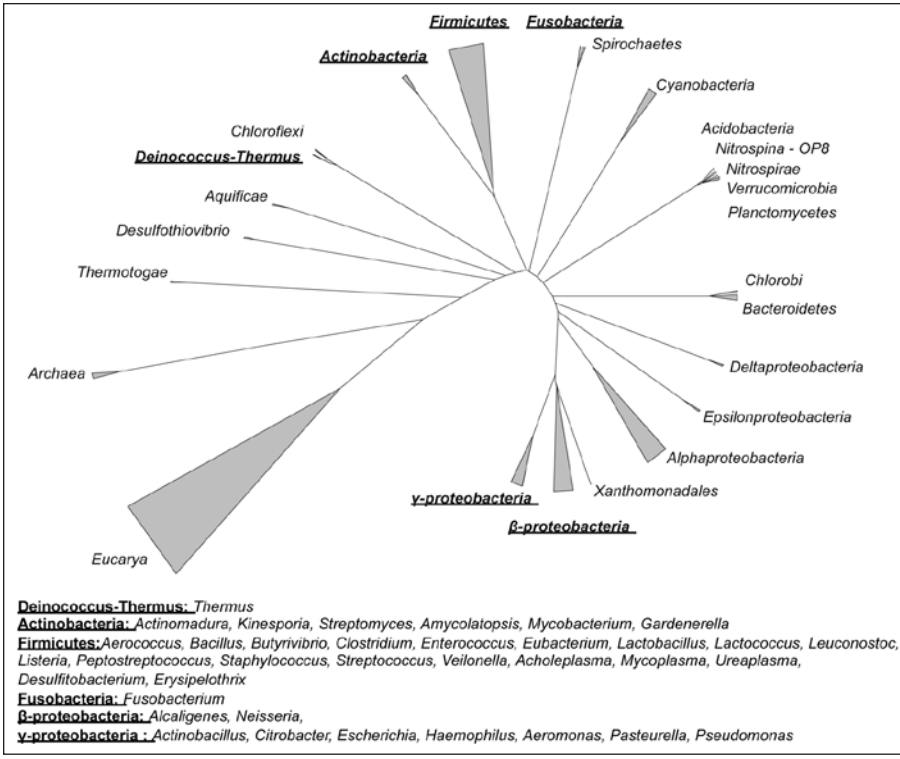

Figure 3. Taxonomic tree of life showing all of the phyla from which members of the Tn*916*/Tn*1545* family have been isolated. The genera within each phylum are shown below. The tree was produced by aligning all Bacterial Domain sequences from the Ribosomal Database Project using the ARB package[59] and the January 2004 database.

the different elements (Fig. 4). In some members of the Tn*916*/Tn*1545* family integration and excision is mediated by large serine recombinase (these proteins do not require an excisonase in the recombination reactions) these include Tn*5397* and Tn*1116*.[8,19]

*Target Sites of Tn*916

The Tn*916* IntTn protein can use multiple target sites and these have been shown to be A:T-rich. A recent study has characterized 123 insertion sites in the genome of *Butyrivibrio proteoclasticus* strain B316[20] and has shown that the consensus sequence TTTTT *TATATA* AAAAA is used (the hexanucleotide in italics is variable and forms the coupling sequences). In addition we have recently performed a similar study in *Clostridium difficile* and have shown a nearly identical consensus sequence based on almost 200 insertion sites in two different strains (Mullany et al., unpublished). Interestingly however Tn*916* has a preferred insertion site in *C. difficile* strain CD37. Here the target insertion site also consists of an A:T-rich region but is preferentially used in this strain.[21]

Conjugation

Knowledge of the specific mechanism of conjugation among the Tn*916* family members is somewhat limited, however early Tn*5* mutagenesis indicated that ORFs *24* to *13* are involved in this process (Fig. 1).[22] However none of the Tn*5* insertions were complemented so polar effects cannot be ruled out. The specific functions of some of these proteins, or homologs of these proteins, have been experimentally proven and are shown in Table 1. Orf20 is a relaxase nicking at the

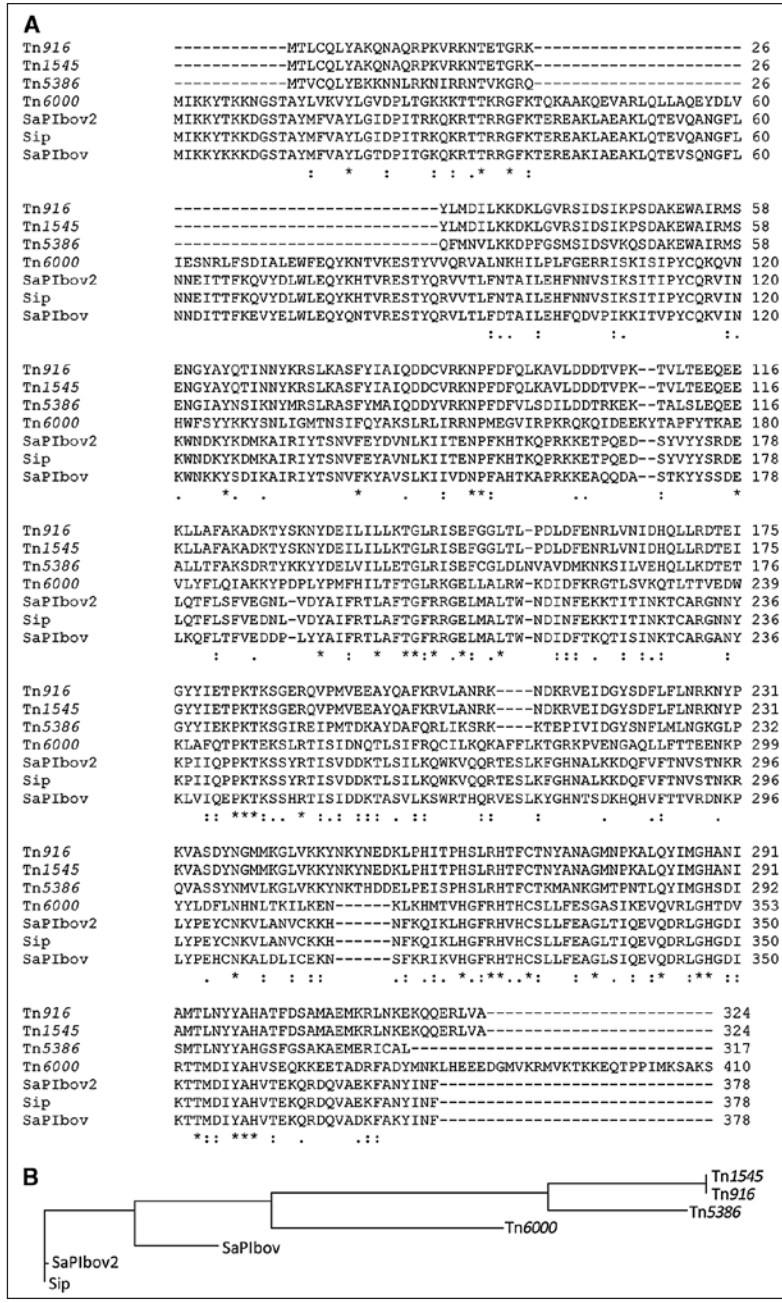

Figure 4. A) Alignment of the integrase genes of Tn*916* (U09422), Tn*1545* (X61025), Tn*5386* (DQ321786), Tn*6000* (FN555436), SaPIbov (AAG29618), SaPIbov2 (AAP55251) and Sip (AAP51267). Sequence accession numbers are shown in brackets above. "*"—identical amino acids; ":"—conserved amino acid substitutions; "."—semiconserved amino acid substitutions. B) A phylogenetic tree of the amino acid sequences is shown. Figures generated using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

origin of transfer (*ori*T), constituting the first step of the conjugation process.[23] Tn*916* IntTn is a specificity factor for this reaction and is responsible for both the strand and sequence selection of Orf20. *Ori*T itself spans a 466 bp region containing a number of inverted repeats and is positioned between *orf20* and *orf21* of the transposon.[24] *Orf18* encodes a putative ArdA homologue which is responsible for the transposon's immunity to DNA restriction modification following conjugation by mimicking the DNA substrate for restriction enzymes. This likely contributes to the broad host range of Tn*916*.[25] The putative product of *orf14* shows some similarity to the NPL/p60 family of proteins which are associated with virulence in *Listeria monocytogenes*.[26,27]

Regulation

Our knowledge of the regulation of Tn*916*/Tn*1545*-like elements is almost completely limited to Tn*916*. Su et al. (28), proposed that the regulatory system of Tn*916* comprises of *orf12*, *orf9*, *orf7* and *orf8* (Fig. 1). This region is conserved in nearly all Tn*916*-like elements which suggest that it is extremely important for the function and / or maintenance of the elements.

The presence of several inverted repeat sequences within *orf*12 are key to the proposed regulatory mechanism. It has been proposed that regulation of Tn*916* involves transcriptional attenuation and is regulated by tetracycline.[28,29] The proposed regulatory region consists of stem-loop structures, 5S:6S and 7:8 followed by a series of uracil residues in the RNA, predicted to be the transcriptional terminators ("T" on Fig. 5). In the presence of tetracycline most ribosomes are inactivated by the reversible binding of tetracycline, resulting in a build-up of charged t-RNA molecules due to a lower rate of protein synthesis. At this stage, a few ribosomes are thought to be protected by the low and basal level of Tet(M). Accumulation of charged t-RNA enables the more rapid translation of *orf12* by the protected ribosomes. This event speeds up the translation by the protected ribosomes, which is normally slow due to the presence of rare codons in *orf12*, and is predicted to allow the ribosome to catch up to the RNA polymerase and therefore prevent the formation of, or destroy, the terminator structures 5S:6S and 7:8. This allows transcription from the promoter upstream of *orf12* to extended into, and through, *tet*(M) and into the downstream genes (Fig. 6). However, in the absence of tetracycline, the ribosome pauses on the leader peptide of *orf12* due to both the shortage of charged t-RNA molecules and the rare codons within *orf12*. This results in the ribosome lagging behind the RNA polymerase allowing the formation of the predicted strong 5S:6S terminator and/or the weaker 7:8 terminator which is predicted to terminate the majority of transcription.

Orf9 is proposed to repress the transcription from the *orf7* promoter Porf7 (Fig. 6). Increased transcription through *tet*(M) and downstream regions will lead to the production of antisense *orf9* RNA which leads to de-repression of Porf7 and increased transcription of Orf7 and Orf8 from the upstream promoter Porf7. Increased transcription from Porf7 will lead to an increase in the translation of Orf7 and Orf8. Orf7 and Orf8 are predicted to upregulate their own transcription from Porf7, thereby providing an amplification of the environmental signal (tetracycline) sensed upstream of *tet*(M). The increase in transcription from Porf7 will lead to an increase in the translation of downstream genes (*xisTn* and *intTn*) promoting excision of the element from the host replicon. In its circular form, transcription continues into the conjugation module (Figs. 1 and 6) presumably promoting transfer.

The regulatory model of Tn*916* has never been experimentally proven although it is fundamentally important. When considering the regulation of Tn*916;* it is not actually dependent on the presence or absence of tetracycline but on the translation rate, where an increased pool of charged t-RNAs is likely to result in the upregulation of Tn*916* genes. This means that any malfunction in the cell's translational apparatus will cause the translation rate to drop and therefore increase the tRNA concentration, which is expected to be deleterious to the cell. Tn*916* should be able to sense this response to cellular distress and respond by activating its own transcription and movement.

Table 1. Functions of orf24–13 of the Tn916 conjugation module

Coding Region	Closest Homolog	Percentage Identity and Coverage (%)	Accession Number	Experimental Evidence of Function	Reference
orf24	*Streptococcus agalactiae* 2603V/R Tn*916* hypothetical protein	100, 97	NP_687949		
orf23	*Streptococcus infantis* ATCC 700779 conjugative transposon protein	99, 99	ZP_08061828		
orf22	*Peptostreptococcus anaerobius* 653-L conjugative transposon protein	99, 99	ZP_06424608		
orf21	*Streptococcus agalactiae* 2603V/R Tn*916*, FtsK/SpoIIIE family protein	100, 99	NP_687949	FtsK/SpoIIIE family protein; required for DNA segregation during cell division	61
orf20	*Streptococcus pneumoniae* putative conjugative transposon replication initiation factor	100, 99	CBW39427	Endonuclease which cleaves Tn*916* at *ori*T	23
orf19	*Streptococcus agalactiae* 2603V/R Tn*916* hypothetical protein	100, 99	NP_687949		
orf18	*Ureaplasma urealyticum* serovar 9 str. ATCC 33175 conjugative transposon protein	100, 99	ZP_03079519	Anti-restriction protein responsible for DNA modification immunity (Ard)	25
orf17	*Streptococcus pneumoniae* putative conjugative transposon membrane protein	99, 99	CBW38812		
orf16	*Enterococcus faecalis* TX0309B putative ATP/GTP-binding protein	100, 99	EFU87609		
orf15	*Peptoniphilus duerdenii* ATCC BAA-1640 conjugative transposon membrane protein	100, 95	ZP_07400188		
orf14	*Streptococcus agalactiae* 2603V/R Tn*916*, NLP/P60 family protein	100, 99	NP_687949	NLP/P60 extracellular lipoprotein	26
orf13	*Streptococcus suis* BM407 membrane protein	99, 99	YP_003028726		

Figure 5. The alternative putative secondary structures that are predicted to form in mRNA of Tn*916*. The stem-loops named 1:2, 3:4 and 5S:6S are mutually exclusive to that of the 5L:6L. The free-energy values are shown in kcal/mol. The gray-shaded area is *orf12*. The Shine-Dalgarno sequences are underlined and labeled. The *tet*(M) start codon is italicised. The structures which include the terminators are labeled (T). Schematic adapted from: Roberts AP, Mullany P. Trends Microbiol 2009; 17:251-8;[4] ©2009 with permission from Elsevier.

Accessory Genes

Most of the Tn*916*/Tn*1545* family of elements possess *tet*(M) which encodes a ribosomal protection protein (RPP) conferring resistance to tetracyclines.[17,28] A number of other accessory genes have also been found among the Tn*916*/Tn*1545* family of transposons (Fig. 2, Table 2). These range from other RPP genes such as *tet*(S) and the efflux gene *tet*(L), a number of macrolide resistance genes such as *erm*(B) and *mef*(E), as well as the mercury resistance gene *mer*(A), the kanamycin resistance gene *aph*A, and the quarternary ammonium compound (QAC) resistance gene *qrg* (Table 2).

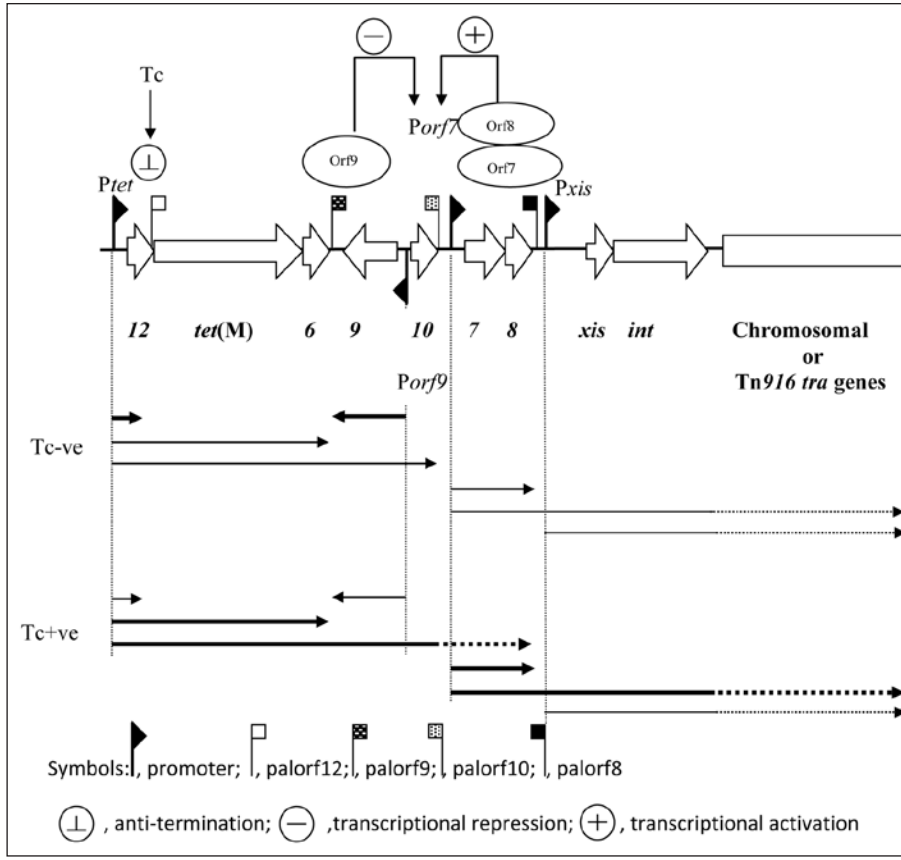

Figure 6. Regulation of expression of the transfer genes within Tn*916* The thick arrows underneath the figure represent the majority of the transcripts, the thin lines represent lower levels of transcription. The dotted lines represent possible basal level, read-through transcripts. In the absence of tetracycline (Tc), most of the transcripts initiated at P*tet* terminate at pal*orf12*, P*orf9* transcribes *orf9* efficiently, whereas P*orf7* directs a low level of transcription through *orf7* and *orf8*. In these conditions, P*orf7* and P*xis* direct a low level of transcription through the transposition-associated and down stream genes. In the presence of Tc, the transcripts initiated at P*tet* read through pal*orf12*, pal*orf9* and pal*orf10* which leads to a decreased transcription of *orf9* and an increased transcription of *orf7* and *orf8*. The resulting overexpression of *orf7* and *orf8* stimulates the activity of P*orf7,* which leads to an increased transcription of the downstream genes. *orf9* could repress the activity of P*orf7*. (Adapted from ref. 60).

*Tn*916 *Can Have Multiple Effects on the Host Genome*

The insertion of Tn*916* into a genome can affect the host in various ways. An insertion within a gene can lead to loss or alteration of gene function. Insertion near genes may lead to polar effects, e.g., in some *E. faecalis* strains Tn*916* has inserted upstream of a hemolysin located on plasmid pAD1 which has resulted in its overexpression.[30] Another mechanism of introducing heritable change in host cells is by transporting non Tn*916* DNA (the coupling sequences) into the cell when the element transfers. In a study Tn*916* was used to create insertional mutations in *Desulfitobacterium dehalogenans*, an anaerobic organism capable

Table 2. Accessory genes found among the Tn916 family of transposons

Transposon	Assessory Genes	Function	Location	Reference
CTn*1*	ABC transporter	unknown substrate	*orf13–7*	56
Tn*1545*	*aphA*	kanamycin resistance	between *orf20–19*	62
Tn*2009*	MEGA *mef*(E)	macrolide resistance	between *orf9–6*	5
Tn*2010*	*erm*(B)	MLS* resistance	between *orf21–20*	63
Tn*2010*	MEGA *mef*(E)	macrolide resistance	between *orf9–6*	63
Tn*2017*	MEGA *mef*(E)	macrolide resistance	between *orf9–6*	64
Tn*2017*	Tn*917 erm*(B)	MLS resistance	between *orf9–6*	64
Tn*3872*	Tn*917 erm*(B)	MLS resistance	between *orf9–6*	65
Tn*5386*	*spa*	subtilisin immunity	in place of *tet*(M)	34
Tn*6000*	*tet*(S)	tetracycline resistance	in place of *tet*(M)	9
Tn*6002*	*erm*(B)	MLS resistance	between *orf20–19*	6
Tn*6003*	*erm*(B)	MLS resistance	between *orf20–19*	62
Tn*6003*	MAS *erm*(B)	MLS resistance	between *orf20–19*	62
Tn*6003*	*aphA*	kanamycin resistance	between *orf20–19*	62
Tn*6009*	*mer*(A)	mercury resistance	upstrem of *orf24*	7
Tn*6079*	*tet*(L)	tetracycline resistance	downstream of *tet*(M)	54
Tn*6079*	*erm*(T)	MLS resistance	upstrem of *orf5*	54
Tn*6087*	*smr*	multidrug resistance	*orf15*	57
Tn*916S*	*tet*(S)	tetracycline resistance	in place of *tet*(M)	66

*MLS; Macrolide, lincosamide and streptogramin

of halorespiration. The transposon mutagenesis generated a relatively high percentage of halorespiration deficient mutants in the strain.[31] This study also revealed that the coupling sequences formed during recombination remained in some of the mutants in the empty target sites following excision of Tn*916*. Therefore, Tn*916* was responsible for the introduction of short fragments of foreign DNA which remained after excision of the element causing deficiency in halorespiration. Another study has shown that Tn*916* had replaced six nucleotides of its target site in *Erysipelothrix rhusiopathiae* with six alternative nucleotides, most likely the coupling sequence.[32] In this case the transposon was responsible for the replacement of six chromosomal

nucleotides (AAACAA) by a six new nucleotides (GTATTA) as a result of its insertion and subsequent excision.

Interactions with Other Mobile Genetic Elements

When investigating the structure and functions of the Tn*916*/Tn*1545* family of transposons, it quickly becomes apparent that one is not looking at a static picture. These elements are constantly evolving and interacting with other MGEs including transposons, plasmids, insertion sequences and introns.

Group II introns have been found inserted into Tn*916*/Tn*1545*-like elements e.g., Tn*5397*,[33] Tn*5386*,[34] Tn*6000*,[9] and Tn*6084*.[10] All of the above are inserted into various *orfs* of the conjugation module of the host elements.

There are multiple insertions in Tn*916*/Tn*1545*-like elements which contain the *erm*(B) gene conferring resistance to macrolide, lincosamide and streptogramin (MLS) antibiotics. Tn*917*[35,36] is found upstream of the recombination module of the elements disrupting *orf9* in SPnRi3*erm*, Tn*3872* and Tn*2017*. In fact the transposition of Tn*917* has been found to be inducible by the presence of erythromycin,[37] much like Tn*916* transfer is thought to be induced by the presence of tetracycline although the molecular mechanisms of induction are different. The macrolide, aminoglycoside, streptothricin (MAS) element[38] has been found in SPnRi3*erm*, Tn*6003* and Tn*1545* within the conjugation module within *orf20*. Macrolide efflux genetic assembly (MEGA) elements[5,39] are also found among the Tn*916*/Tn*1545* family. MEGA, which includes the *mef*(E) efflux gene, has been found in the regulatory region between *orf6* and *orf9* of Tn*2009*, Tn*2010* and Tn*2017* (Fig. 2).

Multiple copies of Tn*916*/Tn*1545*-like elements have been found in various genomes and mobilisation of other Tn*916*/Tn*1545*-like elements has been shown to occur.[40] A recent study describes the presence of three highly similar elements (Tn*6085*a, Tn*6085*b and Tn*6084*) which are all found in one strain, *E. faecium* C68.[10] Interestingly the presence of three transposons does not significantly increase the organism's resistance to tetracycline. In a previous study by the same group, the presence of two related elements in the same strain (Tn*916* and Tn*5386*) resulted in the deletion of a large 178 kb genomic fragment suggesting interaction between the elements.[41] Further investigations indicated that excision of Tn*5386* was catalyzed by the Tn*916* integrase, IntTn, resulting in the simultaneous excision of both elements and the region between them. Another study investigating the target site of Tn*5397* demonstrated that introduction of Tn*916* to strains already containing Tn*5397*, resulted in its loss in > 95% of cases, presumably due to *trans* acting factors from the other element.[42]

Variations on the Tn*916* Theme

*Tn*5397 *from* Clostridium difficile

Tn*5397* was originally identified in *Clostridium difficile* and has subsequently been found or transferred into *E. faecalis*,[43,44] *B. subtilis*,[45] and an oral *Streptococcus* sp.[46] A Tn*5397*-like element; Tn*1116*, has also been discovered in *Streptococcus pyogenes*.[19]

Instead of the tyrosine integrase and the excisionase genes Tn*5397* encodes a large serine recombinase; TndX which catalyzes recombination (Fig. 2).[42] This protein is related to the TnpX resolvase found in the chloramphenicol resistance encoding *Clostridium perfringens* and *Clostridium difficile* transposons, Tn*4451* and Tn*4453* respectively.[42,47] Copies of Tn*5397* are always flanked by a direct repeat of a GA dinucleotide, during excision endonucleolytic staggered cuts, mediated by TndX, occur at the GA leading to G/C and A/T Crick and Watson base pairing at the joint of the circular form.[42,43] Upon excision the target sequence is also regenerated.

The regulation of Tn*5397* is subtly different to that hypothesized for Tn*916*. While the ORFs *orf7* and *orf*8 of Tn*5397* and Tn*916* are homologous and the promoters upstream of *orf7* (Porf7) and *tet*(M) are almost identical, an 88-bp deletion in Tn*5397* effectively removes *orf*12 replacing this region with two alternative ORFs; *orf25* and *orf26*.[48] This deletion results in the disruption of the 5S:6S terminator (Fig. 5) which is predicted to be crucial for the regulation of *tet*(M) in

Tn*916*.[28] Despite these differences, we have shown, both at the phenotypic and genotypic level, that the expression of tetracycline resistance is still inducible (Roberts et al., unpublished). An alternative hypothesis for the regulation of Tn*5397* is currently being tested.

The other major difference between Tn*5397* and Tn*916* is that Tn*5397* has a group II intron inserted in the 3′ end of *orf14*. This intron has been shown to splice out of the pre-mRNA,[33] however splicing is not a prerequisite for conjugal transfer as Tn*5397* containing a mutant intron (with a kanamycin resistance gene inserted into the reverse transcriptase) incapable of splicing, could still transfer from *B. subtilis* to *C. difficile*. As the intron has inserted close to the 3′ end of *orf14* the interrupted gene can presumably still produce a functional protein.[33]

*Tn*6000 *from* Enterococcus casseliflavus

Tn*6000* was originally isolated from a cynomolgus monkey in a study investigating the microbiological effects of amalgam fillings.[49] Tn*6000* encodes a tyrosine integrase; Int6000 which is homologous to Int (42% identical) and Sip (41% identical), the integrases from the bovine staphylococcal pathogenicity islands SaPIbov and SaPIbov2, respectively (Fig. 4).[9,50] It has been shown that the element is flanked by perfect 18 bp direct repeats which are also found in the target site as well as the circular form.[18] This is also the case in SaPIbov2, although the 18bp sequences are different.

Tn*6000* contains some insertions and additions likely derived from diverse sources. Upstream of the conjugation region are a group of five genes of which four are predicted to be involved in restriction/modification and anti-restriction (Fig. 2). These genes are in addition to the Tn*916* anti-restriction gene *orf18* which Tn*6000* also possesses meaning that five of the 29 predicted ORFs are likely to be involved with protecting DNA against restriction enzymes. Next, there is an insertion of a fragment of DNA that shares nucleotide identity and gene order to a region of the virulence-related locus (*vrl*) from *Dichelobacter nodosus*, the causative agent of ovine foot rot.[51] The *vrl* is a 27.1-kb genomic island associated with more virulent strains of *D. nodosus*. It has also been found in *Desulfococcus multivorans*, indicating that it has undergone horizontal gene transfer. The *vrl* is hypothesized to undergo horizontal gene transfer possibly mediated by a bacteriophage such as DinoHI.[52] In Tn*6000*, the genes *vap* and *hel* (Fig. 2) are in the same order as *vrlR* and *vrlS*, a virulence-associated protein and a DEAD helicase of the Super-family 2 from *vrl*. The proteins Vap and Hel are 35% and 36% identical to VrlR and VrlS, respectively. The DEAD-DEAH helicases are involved in ATP-dependent unwinding of nucleic acids and may have a role in the conjugation process of Tn*6000*.

Finally, there is a group II intron present in exactly the same place as the one present in Tn*5397*. These two group II introns are > 99% identical to each other at the nucleotide level. It is therefore likely that the progenitors of one of these elements has previously inhabited the same cell as the other and acquired the intron, either by a retro-homing mechanism or by homologous recombination.

*Tn*6079 *from* Streptococcus gallolyticus

Tn*6079* was recently isolated from a fecal metagenomic fosmid library of a one month old healthy infant boy.[53] It is a composite transposon (28872 bp) carrying both tetracycline and erythromycin resistance genes (Fig. 2). The sequence and overall structure of Tn*6079* is highly similar to putative Tn*916*-like transposons detected in *S. gallolyticus*-like strains, and flanking sequences from the fosmid insert of Tn*6079* were used to assign the original host of the fragment to species level.[53]

Tn*6079* is located at the 3' end of a gene predicted to encode protein L33 from the ribosomal 50S subunit. The element contains complete Tn*916*-like conjugation and recombination modules, but in the regulation module only *orf12* and *orf5* is present (Fig. 1 and 2). Regarding accessory genes, in addition to *tet*(M), Tn*6079* carries another tetracycline resistance gene, *tet*(L) (Table 2) predicted to encode an efflux protein and in addition it carries an erythromycin resistance gene, *erm*(T). The *tet*(L) gene is located just downstream of *tet*(M) and is closely linked to plasmid recombination/mobilization (pre/mob) and replication (rep) genes. Next to this, *erm*(T) is surrounded by IS*1216* transposase sequences. Thus, apparently Tn*6079* has evolved by the integration of different MGEs.

Comparison of sequence and structure of Tn*6079* and corresponding MGEs detected in other *S. gallolyticus* strains[54,55] showed that the element with *erm*(T) and IS*1216* genes most likely was introduced into Tn*6079* by intraspecific genetic exchange.[53] Another accessory gene predicted to encode a cell-surface protein is located just upstream of *orf24*. This gene is highly similar to genes present in the end of CTn*1* from *C. difficile* 630 and Tn*5386* from *E. faecium*.[34,56] Finally, two hypothetical genes with unknown functions are present on both sides of *orf21*.

*Tn*6087 *from* Streptococcus oralis

Tn*6087* was isolated from *Streptococcus oralis* cultured from pooled saliva collected from healthy volunteers.[57] Its architecture is much like that of Tn*916* with the same functional modules and identity in both the regulation and recombination genes. The Tn*6087 tet*(M) is more similar to that found in an *E. faecalis* Tn*916*-like conjugative transposon (DQ223248) than the one in Tn*916* (U09422). The main differences are found in the conjugation region where a number of the ORFs are truncated and a 3 kb insertion is found within *orf15* (Fig. 2).

The insertion within *orf15* was found to be a composite transposon consisting of a QAC resistance gene (*qrg*) and a gene predicted to encode a hypothetical protein flanked by two nearly identical IS*1216* sequences. The Qrg protein sequence showed some limited identity (46–57%) to known small multidrug resistance (SMR) proteins. SMR proteins are known to increase resistance to QACs, and the MIC to cetyltrimethyl ammonium bromide (CTAB) was found to be higher than expected (64 μg/ml) in the *Streptococcus oralis* isolate. To determine whether the increase in resistance to CTAB was due to the presence of *qrg*, the gene was mutated by allelic replacement with a chloramphenicol resistance gene. Resistance to CTAB was indeed found to be lower (16 μg/ml) in the *qrg* deficient mutant, and was restored when the mutant was complemented with *qrg* on a plasmid (32 μg/ml). To investigate how widespread the novel *qrg* was, the gene was amplified from eight metagenomic DNA samples. One sample extracted from pooled saliva and pooled faecal samples taken from volunteers from four European countries.[58] All samples were found to be positive for the novel *qrg* gene and sequence analysis of the amplicons showed at least 97.81% identity to the Tn*6087 qrg*.

PCR analysis demonstrated that recombination occurred in this region resulting in a range of different molecules including the excision of the entire 3 kb composite transposon and a circular form of the *qrg* region and one of the IS elements was also detected (Fig. 7). As in the case of Tn*6079*, it seems likely that the IS*1216* elements were able to mobilise genes foreign to Tn*916* and insert them into this MGE thereby facilitating their spread.

Tn*6087* also differs from Tn*916* in that it has not been shown to have the ability to transfer by conjugation. Single nucleotide polymorphisms in the Tn*6087* sequence within the conjugation module resulted in a number of truncations within these ORFs: specifically in *orf24*, *orf20*, *orf16*, and *orf15*. Furthermore, the presence of the composite transposon within *orf15* was also likely to have an effect on conjugation ability. However, Tn*6087* could be transformed into another *Streptococcus* sp.[57]

Conclusion

The Tn*916*/Tn*1545* family of transposons is diverse and ubiquitous among many bacterial genera. Nearly all members of the family encode tetracycline resistance, but some also have resistance to other antimicrobial agents, such as macrolide antibiotics and some antiseptics. These traits give the host an advantage in certain environments, and importantly in the healthcare setting. Some of the Tn*916*/Tn*1545* family of elements have a composite structure which includes smaller mobile elements within a larger Tn*916*/Tn*1545*-like structure which are also mobilisable. Furthermore, the presence of these elements can have a number of effects on the host genome, from interruptions to genes to the deletions of large parts of the genome. All of the above traits make the Tn*916*/Tn*1545* family of elements important players in bacterial genome evolution. Their ability to modify their host's genotype and phenotype makes further study of these MGEs of high importance.

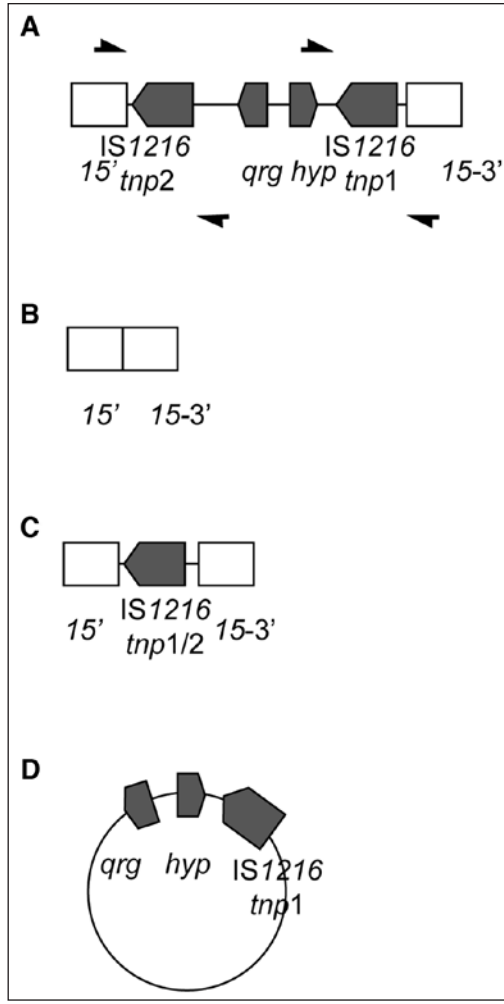

Figure 7. A schematic representing the four forms of the *qrg* gene and IS*1216* insertion found in Tn*6087*. A) The entire 3124 bp insertion within *orf15*; B) the entire insertion excised from *orf15*; C) only a chimeric form of the two IS*1216* sequences remaining; D) and a circular molecule consisting *qrg*, the hypothetical protein and the IS*1216* 1 sequence. The forms described were obtained by sequencing amplicons resulting from reactions using the primers shown in (A).

Questions for the Future

The major gaps in our understanding of Tn*916* and the members of its family lie within the regulation and conjugation modules of the elements. We have only minimal knowledge of the genes involved in conjugation which is one of the most important characteristics of these elements. There is also evidence that some of the genes within the regulation module are affected by the presence of tetracycline in the cell's environment which trigger the transfer of the element. It would also be interesting to investigate how the cells detect the presence of the antibiotic. We have described a high level of plasticity among the members of the Tn*916*/Tn*1545* family, with more being discovered all the time.

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