Comparison of Tn5397 from Clostridium difficile, Tn916 from Enterococcus faecalis and the CW459 tet(M) element from Clostridium perfringens shows that they have similar conjugation regions but different insertion and excision modules

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Comparative analysis of the conjugative transposons Tn5397 from Clostridium difficile and Tn916 from Enterococcus faecalis, and the CW459 tet(M) element from Clostridium perfringens, has revealed that these tetracycline-resistance elements are closely related. All three elements contain the tet(M) resistance gene and have sequence similarity throughout their central region. However, they have very different integration/excision modules. Instead of the int and xis genes that are found in Tn916, Tn5397 has a large resolvase gene, tndX. The C. perfringens element encodes the putative Int459 protein, which is a member of the integrase family of site-specific recombinases but is not closely related to Int from Tn916. Based on these studies it is concluded that the clostridial elements have a modular genetic organization and were derived independently from distinct mobile genetic elements.

Keywords: conjugative transposons, gene transfer, antibiotic resistance, mobile elements

INTRODUCTION

Conjugative transposons are mobile DNA elements that encode all the necessary functions for intracellular transposition and intercellular conjugation. They are found in a wide variety of both Gram-positive and Gram-negative bacteria and are important in the dissemination of antibiotic-resistance genes (Scott & Churchward, 1995). The first conjugative transposon identified, and the most extensively studied, is the 18 kb element Tn916 from Enterococcus faecalis strain DS16 (Franke & Clewell, 1981). Tn916 and the closely related element Tn1545 from Streptococcus pneumoniae (Caillaud et al., 1987; Courvalin & Carlier, 1987) form the basis of a family of conjugative transposons that have an extremely broad host range (Rice, 1998). All of the members of this family carry the tetracycline–minocycline-resistance gene, tet(M) (Burdett, 1991); many also carry genes encoding resistance to other antimicrobial agents.

Conjugative transposition of members of the Tn916 family involves excision from the donor genome, circularization of the element, and then transfer to a new host and subsequent insertion into a new target site. Transposition requires the products of the transposon-encoded int and xis genes, which encode Int, a site-specific recombinase of the integrase family, and Xis, respectively (Poyart-Salmeron et al., 1990). Both Int and Xis are essential for excision but only Int is required for integration (Senghas et al., 1988; Poyart-Salmeron et al., 1990; Lu & Churchward, 1995; Rudy et al., 1997; Marra & Scott, 1999). Int-mediated excision of Tn916 is similar to the excision of λ prophage DNA as it utilizes a mechanism that involves staggered cuts at the ends of the element, circularization, and subsequent transfer to a new host. However, the recombination sites of λ are...
homologous, unlike those of Tn916 (Caparon & Scott, 1989).

Members of the Tn916 family also encode their own conjugal transfer. Analysis of the complete DNA sequence of Tn916 reveals open reading frames (ORFs) that encode putative polypeptides with sequence similarity to proteins known to be involved in conjugation (Flannagan et al., 1994). For example, Orf18 has similarity to the anti-restriction protein Ard of plasmid ColIb-P9 and Orf23 is related to the MeeB mobilization protein of plasmid ColE1. A functional oriT site is also present between orf21 and orf20 (Jaworski & Clewell, 1995).

Work in our laboratories has concentrated on determining the molecular basis for tetracycline resistance in the anaerobic pathogens Clostridium difficile and Clostridium perfringens. In C. difficile, resistance is encoded by the conjugative transposon Tn5397, which mediates transfer between C. difficile strains and to and from Bacillus subtilis (Mullany et al., 1990). DNA hybridization analysis and partial DNA sequence analysis have shown that Tn5397 contains the tet(M) gene and is closely related to Tn916. However, Tn5397 differs from Tn916 in that it contains a Group II intron inserted into orf14 (Mullany et al., 1996) and has different sequences at its ends (Wang et al., 2000a).

In C. perfringens, tetracycline resistance is the most common antimicrobial resistance phenotype (Lyras & Rood, 1996). In most strains, this resistance is non-transferable; however, conjugative transfer of tetracycline resistance is not uncommon (Rood, 1983; Abraham et al., 1985). Transfer is always associated with large conjugal plasmids that are either identical to, or closely related to, the prototype R-plasmid, pCW3 (Abraham et al., 1985; Abraham & Rood, 1985). pCW3 carries the well-characterized tetracycline-resistance determinant Tet(P), which comprises two tetracycline-resistance genes, tetA(P) and tetB(P) (Sloan et al., 1992). The tetA(P) gene encodes a 46 kDa protein that mediates active efflux of tetracycline from the cell. The tetB(P) gene encodes a putative 72-kDa protein that has significant similarity to Tet(M)-like tetracycline-resistance proteins.

Hybridization and PCR analysis of a large number of conjugal and non-conjugative tetracycline-resistant C. perfringens isolates has shown that they all carry the tetA(P) gene. Most (93%) of these isolates carry a second tetracycline-resistance gene, with 53% carrying a tetB(P) gene and 40% carrying a tet(M)-like gene (Lyras & Rood, 1996). No isolates have been detected that carry both the tetB(P) and the tet(M)-like genes. Conjugal transfer of the latter gene has not been demonstrated in C. perfringens.

In this paper, we compare the complete nucleotide sequences of Tn5397, Tn916 and the partial sequence of a tet(M)-like element from C. perfringens strain CW459. The results show that all three elements are closely related but have different excision modules. The comparison of these three elements provides valuable insights into the evolution and dissemination of conjugal transposons in Gram-positive bacteria.

METHODS

Bacterial strains and culture conditions. All Escherichia coli strains were derivatives of DH5α (Life Technologies) or DH5αMCР’ (Gibco-BRL) and were grown at 37 °C in 2xYT (Sambrook et al., 1989) or LB medium (Sambrook et al., 1989) in the presence of 100 µg ampicillin ml⁻¹, 10 µg tetracycline ml⁻¹ or 10 µg minocycline ml⁻¹. C. difficile strain 630 was grown in Brain Heart Infusion (BHI) broth or agar (Oxoid) at 37 °C in an anaerobic chamber (Don Whitley Scientific) in an atmosphere of 80% N₂, 10% H₂ and 10% CO₂ in the presence of 10 µg tetracycline ml⁻¹ or 10 µg erythromycin ml⁻¹. C. perfringens strain CW459 was grown at 37 °C in fluid thioglycollate medium (Difco) or trypticase peptone glucose broth (Rood et al., 1978b) or on nutrient agar or BHI agar in an atmosphere of 80% N₂, 10% H₂ and 10% CO₂ in an anaerobic jar (Oxoid). Tetracycline (5 µg ml⁻¹) was added when appropriate.

Molecular techniques. Plasmid DNA was isolated from E. coli using the Qiagen Miniprep kit or the High Pure Plasmid Isolation Kit (Boehringer Mannheim) in accordance with the manufacturers’ instructions or using a modified small-scale mini alkaline/lysis/PEG precipitation procedure (Applied Biosystems). Genomic DNA was prepared from C. difficile by using the Puregene Gram-positive genomic DNA isolation kit (Flankgen) and from C. perfringens using dye-buoyant gradient ultracentrifugation at 26000 g for 20 h at 20 °C (Abraham & Rood, 1985).

A genomic library of C. perfringens strain CW459 (Rood et al., 1978a) was constructed by digesting purified chromosomal DNA with PstI and ligating it to PstI-digested pSU39 (Bartolome et al., 1991). After overnight ligation at 14 °C, the DNA was introduced into rubidium chloride competent E. coli DH5α cells (Sambrook et al., 1989). The library was screened on media containing tetracycline (10 µg ml⁻¹). Recombinant clones that were tetracycline resistant were screened for minocycline resistance (10 µg ml⁻¹). The recombinant plasmid pJIR1470 was found to confer resistance to both tetracycline and minocycline.

DNA sequencing and analysis. DNA sequencing was carried out using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit on an Applied Biosystems Perkin Elmer 310 Genetic Analyser or an Applied Biosystems 373 DNA Sequencer. DNA sequence analysis was carried out using the DNA Sequencer (Hitachi), NCBI tools (http://www.ncbi.nlm.nih.gov/), European Bioinformatics Institute tools (http://www.ebi.ac.uk/), Sequed (Applied Biosystems), Sequencer 3.0 Software (Gene Codes Corporation) and GeneJockey (Biosoft) programs.

Dot blot hybridization analysis. Plasmid DNA from E. coli and chromosomal DNA from C. perfringens and C. difficile were applied to Hybond-N+ nylon membranes (Amersham) without prior treatment (Sambrook et al., 1989) using a dot blot apparatus (Minifold SRC 96; Schleicher & Schuell). Analysis of dot blots was performed using the DIG DNA Labelling and Detection Kit (Boehringer Mannheim) in accordance with the manufacturer’s instructions. Hybridization was carried out at 65 °C in 5xSSC (0.75 M NaCl, 0.075 M sodium citrate pH 7.0) with subsequent washes of 2x15 min at 65 °C in 0.1xSSC, 0.1% (w/v) SDS. Digoxigenin-11-dUTP-labelled probes were amplified by PCR from Tn916 template DNA and spanned the length of the Tn916 transposon (Fig. 1).
RESULTS AND DISCUSSION

The Tet(M) determinant from *C. perfringens* strain CW459 is located on a Tn916-like element

Previous studies have identified *C. perfringens* strains that carry tet(M) genes (Lyra & Rood, 1996). The tet(M) determinant from one such strain, CW459, was cloned into the plasmid vector pSU39 to generate pJIR1470. Restriction endonuclease digestion showed that this plasmid contained a 28-1 kb PstI insert and DNA hybridization confirmed that it contained the tet(M) gene (data not shown). To see if pJIR1470 carried homologues of other Tn916-encoded genes, the plasmid was hybridized with a series of separate PCR-derived DIG-labelled probes that together encompassed most of Tn916 (Fig. 1). *C. difficile* strain 630, which carries Tn5397, *C. perfringens* strain JIR4229 (Awad & Rood, 1997), which carries a chromosomal copy of Tn916, and pAMI120, an *E. coli* plasmid carrying Tn916, were included as positive controls. The results (Fig. 1) showed that pJIR1470 hybridized to each of the Tn916-derived probes, suggesting that a complete Tn916-like element was present in this plasmid and consequently in strain CW459. However, since this strain is non-conjugative (Rood et al., 1978a), and no evidence for the transposition of its tet(M) gene has been obtained, this region was designated the CW459tet(M) element but not given a formal transposon designation. As expected, *C. difficile* strain 630 also hybridized to these probes (Hächler et al., 1987).

Comparison of the overall genetic organization of Tn5397, Tn916 and CW459tet(M)

Previous studies involved the sequence analysis of the first 328 bp of the left end and the last 2442 bp of the right end of Tn5397 (Wang et al., 2000a). The remaining sequence of Tn5397 was determined on both strands by primer walking on the plasmids pPPM5.3 and pPPM1.6 (Mullany et al., 1996). Gaps in the sequence were filled by designing PCR primers based on the Tn916 sequence (GenBank accession no. U90422) and the unpublished *C. difficile* sequence (http://www.sanger.ac.uk/Projects/C difficile/). To avoid PCR-induced errors, each PCR product was amplified and sequenced at least three times. The results showed that Tn5397 was 20658 bp in length (GenBank accession no. AF333235) and had 21 potential ORFs that had a suitably spaced ribosome-binding site upstream of the putative start codon. The ORF within the Group II intron (Mullany et al., 1996) and tndX (Wang et al., 2000a; Wang & Mullany, 2000) have been described previously. Seventeen of the ORFs were very similar to corresponding ORFs from Tn916 (Flannagan et al., 1994) and were labelled accordingly (Fig. 2).

The CW459tet(M) element was sequenced on both strands by primer walking on a pJIR1470 template. An 8691 bp region including the tet(M) gene and the right end of the element was sequenced (GenBank accession no. AF329848). The G + C content of this region was 35.9 mol% compared to 38.3 mol% for Tn5397 and 38.8 mol% for Tn916. Since CW459tet(M) is not a functional mobile element, the left end of the element was not completely sequenced. The overall structure of both clostridial elements is very similar to that of Tn916 (Fig. 2) although there are numerous insertions, deletions and rearrangements, both within ORFs and in the intergenic regions. This finding also applies to the incomplete single-stranded sequence that was obtained from the left end of the CW459tet(M) element (data not shown). Nine of the ten sequenced CW459tet(M) ORFs were closely related to those of Tn916 and were named accordingly (Fig. 2). A comparison of each of these putative protein products is shown in Table 1.

**Tn5397, CW459tet(M) and Tn916 have different excision/insertion modules**

The most notable differences between the three elements occurred at the extreme right end, where both clostridial elements diverged from Tn916 and from each other (Fig. 2). In these elements, divergence from Tn916 occurs within orf5, suggesting that this region is a recombinational hotspot. Tn5397 only has the 3' end of orf5 but is still capable of conjugative transfer, providing evidence that orf5 is not essential for the transfer of this element.

In Tn916, the int and xis genes, the products of which are essential for excision/insertion, are located to the right of orf5 (Fig. 2). In the clostridial elements, these genes have been replaced with other genes that encode different site-specific recombinases. Database searches with the putative protein encoded at the right end of the CW459tet(M) element revealed 21–28% identity to several bacteriophage or transposon-encoded integrases from Gram-positive bacteria (GenBank accession nos AAA85500, AAC48867, A69774 and C53205) and 17.5% identity to Int from Tn916. Therefore, the CW459-derived ORF was designated int459 (integrase CW459). A comparison of Int459 with other members of the integrase family showed that the conserved integrase motif (Abremski & Hoess, 1992) was present in the putative Int459 protein. This protein may have played a role in the transposition and introduction of the CW459tet(M) element into CW459. However, since conjugative transfer of the CW459tet(M) element is not observed, it appears that either this protein is not produced, is not functional, or is not sufficient on its own for transposition. As observed for the transposition of Tn916 (Jaworski et al., 1996; Marra & Scott, 1999), the excision, and therefore transposition, of the CW459tet(M) element may require additional proteins, such as an excisionase, which are not present in this element.

An additional 170 bp incomplete ORF was identified at the right of the sequenced CW459tet(M) region and was designated gmp because the deduced protein product had 70% identity over 47 amino acids to a GMP synthetase protein from *B. subtilis* (Mantsala & Zalkin, 1992). Since this enzyme is a housekeeping protein we
concluded that it was outside the CW459tet(M) element. Therefore, the right terminus of the element appeared to be located within the 67 bp region between int459 and gmp (Fig. 2). Although this region contained several repeat sequences, it did not have any similarity to the ends of Tn916, Tn5397 or any other known conjugative transposons.

Downstream of orf8 in Tn5397 (Fig. 2) is the tndX gene, which encodes a 61-5 kDa protein, TndX (Wang et al., 2000a), that has 37% identity to TnpX, a large resolvase from the C. perfringens transposon Tn4451 (Bannam et al., 1995) and the C. difficile transposon Tn4453a (Lyra & Rood, 2000). Functional analysis has shown that TndX is involved in the excision and integration of Tn5397 in C. difficile. The tndX gene ends 10 bp before the right terminus of Tn5397 (Wang et al., 2000a; Wang & Mullany, 2000).

The Tn5397 and CW459tet(M) elements do not have the tet(M) leader peptide orf12

Just upstream of the tet(M) gene in Tn916 there is a small ORF, orf12, which encodes a tet(M) leader peptide that is involved in the regulation of tet(M) expression by
Table 1. Comparative analysis of the Orf14–Orf5 region of the CW459tet(M) element, Tn916 and Tn5397

<table>
<thead>
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<th>ORFs</th>
<th>Tn916</th>
<th>CW459</th>
<th>Tn5397</th>
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<td>100.0</td>
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<td>52.7</td>
<td></td>
</tr>
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<td>99.7</td>
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<td>87.1</td>
<td></td>
</tr>
<tr>
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<td>100.0</td>
</tr>
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<td></td>
</tr>
<tr>
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</tr>
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<td>53.2</td>
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<tr>
<td>Tn5397</td>
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<td>59.6</td>
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</table>

* Over 23 amino acids
† Over 62 amino acids.

| Percentage identity is shown. |

<table>
<thead>
<tr>
<th></th>
<th>Tn916</th>
<th>CW459</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>Tn5397</td>
<td>90.5</td>
<td>90.5</td>
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The Tet(M) proteins from Tn916 and the CW459tet(M) element are more closely related to each other than to Tet(M) from Tn5397

The deduced amino acid sequence of the Tet(M) protein from the CW459tet(M) element was almost identical to the Tet(M) protein from Tn916 (Su et al., 1992; Flannagan et al., 1994) but was less closely related to the Tet(M) protein from Tn5397 (Table 1). Comparative analysis was also performed with other Tet(M) proteins from a variety of bacterial genera. A high degree of sequence identity (91–99%) was observed (Fig. 4). Tet(M) from Tn5397 had slightly higher identity (93%) to the Tet(M) protein from Tn1545 (Martin et al., 1986) than to the Tet(M) protein from CW459 or Tn916 (Table 1). These results suggest that the acquisition of the CW459 resistance determinant from a precursor carrying a Tet(M) gene was a relatively recent event and that the Tet(M) genes in CW459 and Tn5397 were not derived from each other but originated from different donors at different times.

The regions between tet(M) and the recombinase genes are similar but not identical to the equivalent region of Tn916

The regions between tet(M) and the recombinase genes are closely related in all three elements (Table 1). However, the CW459tet(M) ORFs are more closely related to those of Tn916 than to Tn5397 (Table 1). Celli & Trieu-Cuot (1998) showed that orfs 7, 8 and 9 encoded putative regulatory proteins. Homologues of these ORFs are present in similar size and arrangement in the clostridial elements (Table 1). The proposed regulatory region in Tn916 includes the palindromes paldorf10a and palorf10b, which are involved in the termination of readthrough transcription from the upstream tet(M) promoter. Similar but not identical palindromes are found in both clostridial elements.

In both Tn5397 and the CW459tet(M) element there was an intergenic region after orf8, followed by the remnants of orf5. In both elements, the N-terminal encoding region of orf5 is missing. In Tn916, the orf8–orf5 intergenic region contains the promoter region for the xis and int genes (Celli & Trieu-Cuot, 1998). The intergenic region in the CW459tet(M) element was similar in size and nucleotide sequence identity (79%) to the equivalent region of Tn916. The xis–int promoter region was present but there were two single base changes. Two regions of dyad symmetry, which have some similarity to the imperfect inverted repeats paldorf10a.
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**Fig. 3.** Comparative DNA sequence analysis of the region just upstream of \( tet(M) \) in \( \text{Tn}5397 \), \( \text{Tn}916 \) and \( \text{CV459} \text{tet(M)} \).
The deduced amino acid sequence of the Orf12 leader and \( tet(M) \) of \( \text{Tn}916 \) is shown in the single letter code underneath the nucleotide sequence. Ribosome-binding sites are underlined and shown in bold. Inverted repeats within \( \text{orf12} \) are underlined or overlined with half arrows. Direct repeats (CCTTTT) associated with the 155 bp deletion region upstream of the \( \text{CW459} \text{tet(M)} \) gene are underlined by half arrows. Direct repeats (CCCAGT) associated with the 88 bp deletion in \( \text{Tn}5397 \) are overlined by half arrows.

**Fig. 4.** Phylogenetic relationship between ribosomal protection proteins. The relationship of ribosomal protection proteins was estimated using the neighbour-joining distance procedure (Thompson et al., 1994). The alignment was constructed using CLUSTAL W (Thompson et al., 1994). The reliability of the branching was assessed using bootstrapping analysis (1000 replications). Statistical significant support (<70%) for a branch is indicated at the node as a percentage. The branch lengths are scaled in proportion to the extent of the change per position as indicated by the scale bar. No correction for multiple substitution was made. The tree showing the evolutionary relationships between these proteins was constructed using TreeView (Rhoderic D. M. Page). The phylogenetic tree was constructed from amino acid sequences from the following proteins: Tet(S) from \( \text{Lactococcus lactis} \) (accession no. CAA63528), Tet(O) from \( \text{Campylobacter jejuni} \) (accession no. AAA23033), Tet(W) from \( \text{Butyrivibrio fibrisolvens} \) (accession no. CAA10975), Tet(T) from \( \text{Streptococcus pneumoniae} \) (accession no. AAA10975), Tet(B) from \( \text{C. perfringens} \) (accession no. AAA10975), and OtrA from \( \text{Streptomyces rimosus} \) (accession no. S18572). Tet(B) and OtrA were used as outgroups as they are the most distantly related ribosomal protection proteins.

and \( p\alpha_l \text{orf4b} \) from \( \text{Tn}916 \), were present. These repeats are believed to be involved in transcriptional termination from the upstream \( \text{orf7} \) promoter (Celli & Trieu-Cuot, 1998). In contrast, the smaller \( \text{orf8–orf5} \) intergenic region of \( \text{Tn}5397 \) has sequence identity (74%) to only part of the equivalent \( \text{Tn}916 \) region. In \( \text{Tn}5397 \), a major deletion has occurred at the beginning of this region. This deletion effectively removes the promoter area of
the missing xis and int genes and the pal_int repeats. To
the right of the remainder of orf5 the sequence of the
clostridial elements diverges dramatically as already
discussed.

**Tn5397 contains homologues of the Tn916
conjugation genes**

As previously described, the 180 bp at the left end of
Tn5397 are not related to Tn916 (Wang et al., 2000a).
The first seven nucleotides of orf24 are also missing.
Following this region, the two transposons are very
closely related (Fig. 2) although several insertions and
deletions are present in Tn5397. The deduced amino
acid sequences of Orfs 23–16 have 90–100% identity to
their Tn916 homologues; Orf15 has 80% identity.
Following orf21 is a 392 bp intergenic region which
contains a sequence that is homologous to the functional
oriT region of Tn916 (Jaworski & Clewell, 1995).
Within the oriT sites of Tn916 and the F plasmid there
is a conserved nick site, 5’-TGGTGTTGG-3’. This site is
identical in Tn5397.

Preliminary sequence analysis of the CW459tet(M)
element indicated that sequences with similarity to much
of the region encompassing the conjugation genes of
Tn916 appear to be present. However, its exact genetic
organization and precise relationship to the equivalent
region in Tn916 remain to be determined. The conserved
oriT nick site is present.

The first ORF of the double-stranded sequenced region
of the CW459tet(M) element, orf15, was only partially
sequenced. However, the amino acid sequence of the
deduced protein had only 21% identity to a corre-
spanding 288-amino-acid region of Orf15 from Tn916
(Flannagan et al., 1994) and 23.9% identity to a corre-
spanding region of Orf15 from Tn5397. By compari-
son with Tn916, Orf15 from both Tn5397 and the
CW459tet(M) element contained C-terminal trun-
cations of 25 and 57 amino acids, respectively. In
Tn5397, the next ORF, orf14, is disrupted by a Group II
intron (Mullany et al., 1996). Neither the CW459tet(M)

element nor Tn916 contains a Group II intron in orf14
(Fig. 2). Comparative analysis of the deduced amino
acid sequences showed that Orf14 (minus the Group II
intron) from Tn5397 is most closely related to Orf14
from Tn916.

**Evolution of Tn5397 and the CW459tet(M) element**

Both Tn5397 and the CW459tet(M) element appear to
consist of four distinct modules that may have originated
from separate mobile genetic elements. The first module
is common although not identical and consists of the
Tn916-like conjugation region (orf23–orf13), which
is present in Tn5397 and most probably in the
CW459tet(M) element. The resistance determinant
comprises the second module, with the tet(M) genes of
Tn916 and CW459 being very closely related and distinct
from that of Tn5397, which is more closely related to the
tet(M) gene from Tn545 (Fig. 4). The third module
comprises the putative regulatory region (orf12–orf8).
The regulatory and resistance modules may overlap, as
orf12 is involved in sensing tetracycline and conse-
quently regulating tet(M) expression. The fourth major
module is responsible for the excision and insertion of
the transposons or their precursors and consists of xis
and int in Tn916, int459 in CW459tet(M) and intX in
Tn5397. Therefore, the three elements have different
excision and integration systems.

Tn916-like sequences have also been found in other
mobile genetic elements. The tet(M) genes of the large
conjugative plasmids of Neisseria gonorrhoeae and
Neisseria meningitidis are associated with Tn916-like
sequences that encompass regions upstream and down-
stream of the resistance determinant (Swartley et al.,
1993). Other studies involving the non-conjugative
multiple antibiotic resistance plasmid pK214 from L.
lactis, which carries resistance determinants for strep-
tomycin, tetracycline and chloramphenicol, have revealed
that the tetracycline-resistance gene, tet(S), is
linked to sequences with similarity to orf7, orf9 and
orf6, which have a similar genetic organization to that
observed in Tn916. This Tn916-like region is linked to
distinct gene regions from three other bacterial species
as well as to numerous insertion sequences (Perreten et
al., 1997; Teuber et al., 1999). Finally, the integrase from
a lysogenic Mycoplasma arthritidis bacteriophage has
greater similarity to Int from Tn916 than to any other
integrase (Voelker & Dybvig, 1998), suggesting a
common evolutionary origin.

There are several hypotheses that may explain the
evolution of the non-functional CW459tet(M) element.
Firstly, it is possible that a modular conjugative trans-
poson may have been initially formed by recombination
events between different mobile genetic elements in
another bacterium and then subsequently transferred to
C. perfringens. Upon insertion into the chromosome,
deletion or rearrangement events may have occurred
which resulted in the loss of mobility of the transposon.

Similar events, resulting in deletions of sections of
Tn916, have been observed in Neisseria (Swartley et
al., 1993). Altered Tn916-like elements, which are no longer
capable of conjugative transfer, have also been detected
in the non-conjugative plasmid pKQ1, from Enter-
ococcus faecium (Fletcher & Daneo-Moore, 1992). Sec-
daely, a composite element may have been formed in C.
perfringens strain CW459 by a recombination event
between a previously acquired, ancestral Tn916 or
Tn916-like transposon and a second mobile genetic
element, which carried an Int459-based excision/
integration system. Alternatively, the recombination
event may have involved a resident element containing
int459 and an incoming Tn916-like transposon. Note
that the conjugative transposition of Tn916 into the
chromosome of C. perfringens from both E. coli and
Enterococcus faecalis has been observed (Kaufmann et
al., 1996). Tn916 has also been shown to transfer into C.
difficile from B. subtilis (Mullany et al., 1991) and
natural isolates of C. difficile that carry Tn916 have
recently been identified (Wang et al., 2000b).
Although direct transfer of genetic information between \textit{C. perfringens} and \textit{C. difficile} has not been demonstrated, closely related transposons have been detected in both species, indicating that genetic exchange whether directly, or indirectly through another bacterial host, can occur (Lyars et al., 1998). The TndX protein, which is involved in the excision and integration of Tn3397, is more closely related to the TnpX resolvase that is responsible for the movement of the \textit{C. perfringens} transposon Tn4451 and the closely related \textit{C. difficile} Tn4453 transposons than to any other resolvase (Wang et al., 2000a; Lyars & Rood, 2000). It has been proposed that one of these elements may have been involved in recombination events with an ancestral Tn916-like element that resulted in the formation of Tn3397 (Wang et al., 2000a).

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