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Mechanism of integration and excision in conjugative transposons

P. Mullany*, A. P. Roberts and H. Wang

Department of Microbiology, Eastman Dental Institute for Oral Health Care Sciences, University College London, University of London, 256 Gray's Inn Road, London WC1X 8LD (United Kingdom),
e-mail: p.Mullany@eastman.ucl.ac.uk

Abstract. Translocation of conjugative transposons proceeds via excision of the element to generate a circular molecule that can then integrate into a new site, which can be in the same or a different cell. This review summarises some of the different mechanisms used for excision and integration of conjugative transposons.

Key words. Tyrosine recombinase; serine recombinase; Tn*916*; Tn*5397*; CTnDOT.

Introduction

The movement of all conjugative transposons investigated so far proceeds via excision of the element to form a circular molecule followed by transfer to a new host and subsequent insertion into a new target site. However, the molecular events underlying these processes differ between conjugative transposons.

Excision and insertion of all the conjugative transposons investigated to date is mediated by a site-specific recombinase. These enzymes fall into one of two families, either tyrosine or serine recombinases, based on evolutionary and mechanistic comparisons. Both families are structurally diverse, including integrases, resolvases, invertases and transposases. To date, the mechanisms of excision and insertion of three conjugative transposons have been investigated in detail. This article summarises our current understanding of these processes.

Mechanism of Tn*916* insertion and excision

Excision and integration of Tn*916* (see fig. 1) requires the integrase protein Int (a tyrosine recombinase) and the excisionase Xis (a small basic protein). Int is required for both excision and integration. Xis greatly stimulates excision and in some hosts is absolutely required for this process. At higher concentrations Xis also inhibits integration [1].

Prior to excision Int generates 5′ protruding staggered endonucleolytic cuts at each end of the element. One strand of DNA is cut five or six bases from the end of the transposon, and the other is cut immediately adjacent to the other end [2, 3]. This generates single-stranded overhangs, which are ligated to form a non-replicative circular molecule. As Tn*916* does not usually duplicate its target on insertion, the two overhangs are not complementary, and the joint between the ends of the transposon in the circular molecule may be a heteroduplex. Prior to transfer to a recipient cell, it is thought that the double-stranded circular form is nicked (at the *oriT* site) and a single strand is transferred to a recipient cell. In the recipient the single strand acts as a template for second-strand synthesis.

Insertion is the reverse of excision; staggered cuts are made at the joint in the circular molecule and at the new target site, strand exchange and ligation occur and the transposon is integrated into its new host molecule. Tn*916* shows preference for a target that has a T-rich region separated from an A-rich region usually by 6 bp. This is also the length of the coupling sequence, which acts as a spacer between the transposon ends in the circular form [4].

Functional analysis of Int and Xis

Int of Tn*916* is related to the phage λ Int protein. In this review, by analogy to the λ system, we will refer to the re-

* Corresponding author.

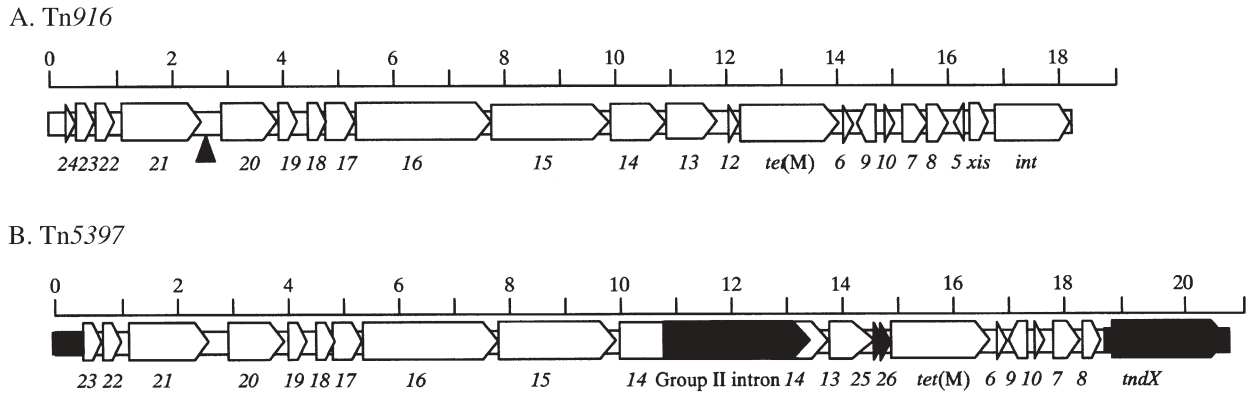

Figure 1. Schematic representation of the structural organization of (*A*) Tn*916* and (*B*) Tn*5397*. The top line shows the scale in kilobase pairs. The pointed boxes represent the open reading frames; the point shows the direction of transcription. The *ORFs* have been named as previously described [16]. The filled triangle indicates the position of the *oriT* in Tn*916*. The regions that are filled in Tn*5397* are unique to Tn*5397* (adopted from [16]).

gion at which recombination takes place as the core sites and the ends of the transposon adjacent to the core sites as arm sites (see fig. 2).

The Int protein of Tn*916* has two domains. The C-terminal domain performs the recombination reactions and binds to core sites at each end of the transposon, which extend into the flanking bacterial DNA. The N-terminal domain binds to the DR2 repeats at each end of the transposon (fig. 2). Therefore it is assumed that during excision of Tn*916* four Int molecules are bound to the transposon ends with the C-terminus of Int bound to each end of the transposon and the N-terminus to the DR2 repeats. This binding of Int results in the transposon ends being brought together: see the review of Scott and Churchward for a diagrammatic representation of this event [3].

Xis is a small basic protein, required for excision of Tn*916*. It binds near the ends of the element near to the N-terminal binding site of Int (fig. 2). DnaseI protection assays with Xis show that it binds to a region which exceeds what was expected based on the size of the protein [1, 5]. This can be explained by postulating that DNA is wrapped around Xis, thus protecting a larger region. Another possibility is that Xis may bind to the DNA in the crook of a static bend. Therefore, Xis may facilitate the binding of Int to DNA by bending the target DNA (but see below for further discussion of this point) or by formation of protein-protein or protein-DNA complexes, or both [1, 5].

Recent work has shown that mutations at the right end of Tn*916* that decrease the binding of Xis result in a higher frequency of excision compared with the wild type, indicating that Xis inhibits excision [1]. In contrast, a mutation in the left end of Tn*916* that interferes with the binding of Xis decreases the frequency of excision, indicating that Xis binding to the left end of the transposon promotes excision. Further DNA-binding experiments demonstrate that Xis has a higher affinity for the left end of the transposon than the right end. Taken together, these results indicate that binding of Xis to the left end of the element is required for excision. However, at higher concentrations Xis binds to the right end, inhibiting excision.

The left-end binding site of Xis is located between the N- and C-terminal binding sites of Int, similar to the organisation in phage λ (fig. 2). This prompted the proposal that as in λ, Xis facilitates binding of Int in Tn*916* and that Xis is required to form the appropriate synaptic

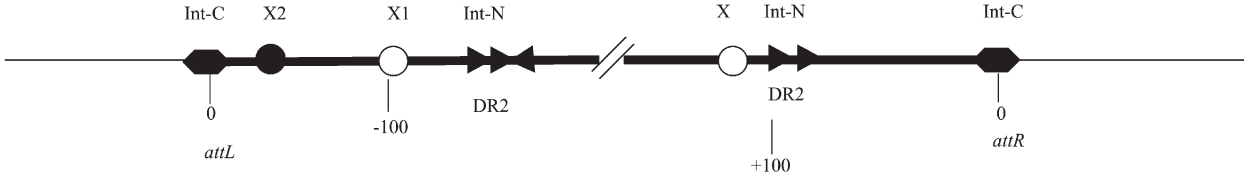

Figure 2. Diagram showing the binding sites of Int and Xis at the left and right ends of Tn*916*. The thick line represents the transposon, and the thin line flanking sequences. The binding site for the C-terminal part of Int at the core region is shown by a hexagon. The binding sites for the N-terminal region of Int in the arm region are shown by a triangle. The binding sites for Xis in the arms of the transposon are shown by a circle. Both ends of the transposon are marked with the number 0, 100 bp into the transposon from the left end is marked –100 and 100 bp into the transposon from the right end is marked +100 (adapted from [3]).

complex. However, in λ Xis facilitates the action of Int by bending the DNA to allow the C-terminal domain of Int to bind to phage DNA. In the Tn*916* system the N- and C-terminal domains of Int are large enough to allow DNA bending to occur without the help of another protein [5]. Therefore, Xis may exert its effect via protein-protein interactions. The repressive effect of Xis at the right end of the transposon may result from it blocking the binding of Int, as the Xis binding site here is adjacent to that of the N-terminal domain binding site of Int.

Further work on the role of other factors in excision of Tn*916* in *Escherichia coli* has shown that the histone-like factor HU greatly enhances excision [5]. However, the integration host factor (IHF) and the factor for inversion stimulation have little effect on excision. This is in contrast to the situation in λ where IHF greatly stimulates excision, but HU has only a modest effect. This probably reflects the fact that Tn*916* is adapted for existence in a wide variety of hosts, and HU proteins are well conserved in prokaryotes [5]. HU probably stabilises the various secondary structures that are required for Int-mediated excisive recombination.

Regulation of insertion and excision in Tn*916*

Conjugation and transposition of Tn*916* is subject to regulation, as uncontrolled movement of the element would be deleterious for both the transposon and the host. Celli

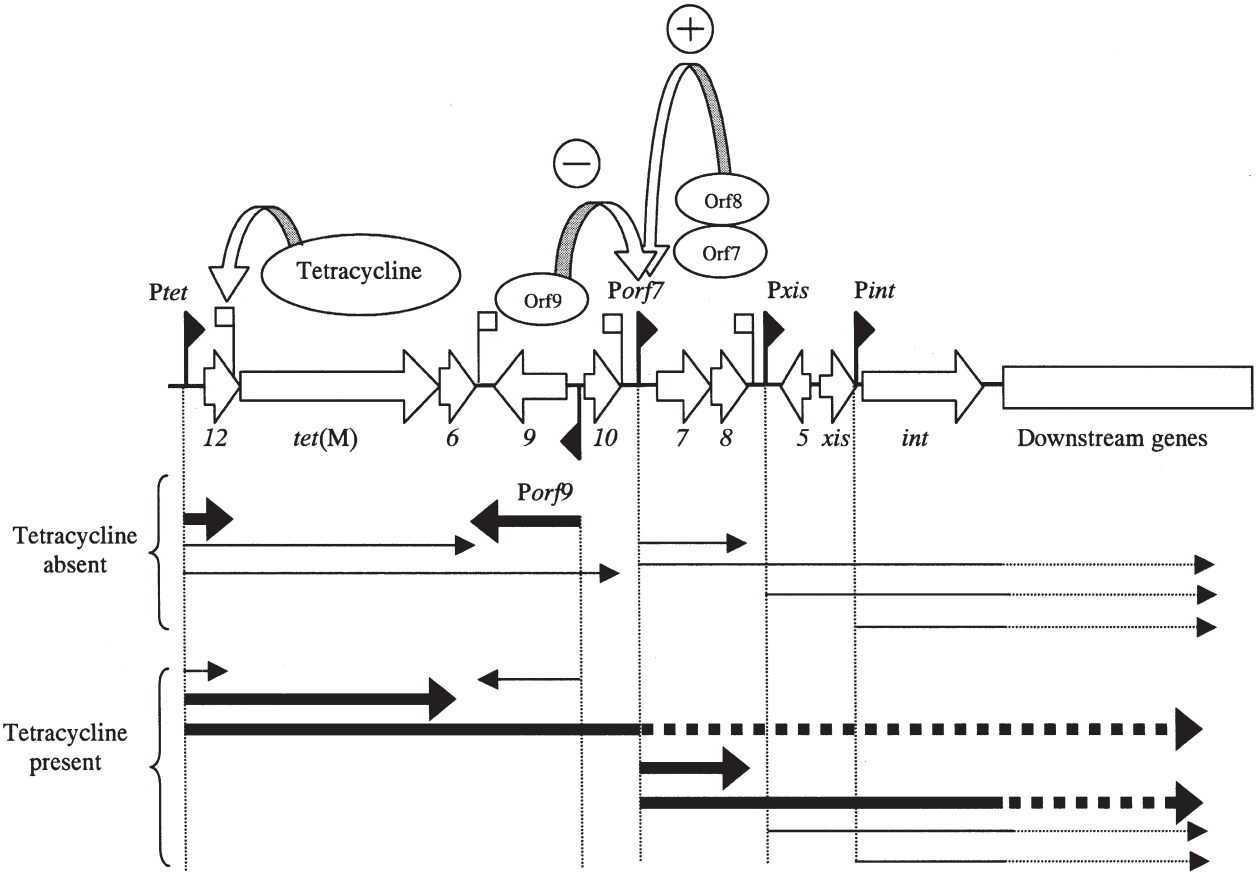

Figure 3. The region of Tn*916* from *orf12* to *int*. The filled triangular flags represent the promoters, with the point of the flag showing the direction of transcription. The unfilled square flags represent palindromic sequences that are believed to act as transcriptional terminators. The circle containing '+' represents transcriptional activation, and the circle containing – represents transcriptional repression. The ORFs are represented by unfilled arrows with the directions of the arrows indicating the probable direction of transcription. The rectangle labeled 'downstream genes' represents host chromosomal genes or the transfer region of Tn*916* if the transposon is in the circular form. The thick arrows below the figure indicate a high level of transcription, the thin arrows a low level of transcription. The dashed arrows represent putative transcription past the terminators. If tetracycline is absent, the majority of transcripts initiating at P*tet* terminate at the palindromic sequences within *orf12*, P*orf9* transcribes *orf9*, which represses transcription at P*orf7*. In these circumstances a low level of basal transcription is observed from this (and presumably P*xis* and P*int*) into downstream genes. When tetracycline is present, a transcriptional attenuation mechanism is thought to allow transcription through the terminator at the end of *orf12*, which results in increased transcription of *tet*(M) and downstream genes. This is believed to reduce the production of *orf9* (possibly by an antisense mechanism), leading to increased levels of transcription from P*orf7* (and possibly P*xis* and P*int*) into the downstream genes (adapted from [6]).

and Trieu-Cuot [6] performed a detailed transcriptional analysis of Tn*916* and showed that transcription of the transfer genes requires excision and circularisation of the element, a process enhanced by tetracycline. Northern blot analysis, promoter fusions and primer extension showed that the key components of this regulatory system are *orf7, orf8, orf9* and *orf12* (figs. 1, 3). It is postulated that the *orf12* region is involved in upregulation of transcription in the presence of tetracycline via a transcriptional attenuation mechanism [7].

There are at least two promoters upstream of *int* and *xis* which produce transcripts that extend into these genes: a relatively weak promoter upstream of *xis,* P*xis*, and a stronger promoter, P*orf7*, which is dependent on the *orf7* and *orf8* gene products, which are upregulated in the presence of tetracycline [6]. There is also a promoter just upstream of *int* but downstream of *xis*, P*int,* which would direct the production of transcripts that include *int* but not *xis* (fig 3).

A plausible model for regulation in Tn*916* is as follows: If tetracycline is absent, the majority of transcripts initiating at P*tet* terminate at the palindromic sequences within *orf12*; P*orf9* transcribes *orf9*, which represses transcription at P*orf7.* In these circumstances a low level of basal transcription is observed from P*orf7* (and presumably P*xis* and P*int*) into downstream genes. When tetracycline is present a transcriptional attenuation mechanism is thought to allow transcription through the terminator at the end of *orf12,* which results in increased transcription of *tet*(M) and downstream genes. This sequence of events is believed to reduce the production of ORF9 (possibly by an antisense mechanism), leading to increased levels of transcription from P*orf7* (and possibly P*xis* and P*int*) into the downstream genes. Xis has an inhibitory effect on insertion, so the directionality (insertion verses excision) of recombination is determined by the relative amounts of Int and Xis. This ratio could be determined by the promoter P*int,* which directs transcription of *int* but not *xis*. However, there is no information available on the regulation of this promoter.

As well as binding to the ends of Tn*916,* Int also binds to its *oriT* sequence [8], which led to the proposal that Int binding to *oriT* would prevent transcriptional readthrough from bacterial promoters into the transfer genes. The promoters P*int,* P*xis* and P*orf7* all have the potential to produce Int, and there is evidence that these promoters are constitutively active in all cells [16]. Therefore, Int should be present in all cells, not just the minority that can act as conjugal donors of Tn*916.* The interaction between Int and *oriT* provides a further level of control in the system and may prevent premature transfer of the element and also prevent excision occurring until the appropriate signals have been received, e. g. the presence of a suitable recipient.

Mechanism of excision and integration in the *Bacteroides* conjugative transposons

The best understood of the *Bacteroides* conjugative transposons is CTnDOT (Whittle et al. this issue). This element contains an integrase gene (*int*) located at one end. The deduced amino acid sequence of Int shows that like Tn*916* Int, it is a tyrosine recombinase related to the λ family [9]. Also, like the Tn*916* system, excision of CTnDOT proceeds via the formation of staggered cuts in the DNA, usually 4–5 bp away from the ends of the integrated transposon. These coupling sequences then become part of the circular form of the element. The Int protein is required and sufficient for integration, but at least one other protein is required for excision. Despite these similarities, there are important differences between the two systems. The CTnDOT element has a number of highly preferred integration sites, which may be due to a 10-bp sequence in the transposon which is identical to a 10-bp sequence adjacent to the insertion site. CTnDOT also differs from Tn*916* in that its transfer efficiency is independent of the site of insertion. Moreover, excision of CTnDOT does not require a small basic protein like Xis, but is dependent upon the product of the CTnDOT-encoded *exc* gene, which is located 15 kb from *int*. The *exc* gene is predicted to encode a protein related to topoisomerase [10]. This is the first time that a topoisomerase-like protein has been shown to be involved in excision. The regulation of CTnDOT is reviewed by Whittle et al. in this volume.

Integration and excision mediated by the serine recombinases

The serine recombinases are a diverse group of site-specific recombinases which mediate recombination via a concerted cleavage and rejoining mechanism. Short-lived phosphoserine links are formed at the 3′ end of a 2-bp sequence, generating a staggered break. In a recent review Thorpe and Smith [11] classified these proteins into five different groups based on their evolutionary, mechanistic and structural relatedness. One of these groups, class V, contains the most structurally and functionally diverse serine recombinases. This class of proteins have also been called the large resolvases due to their homology in the N-terminal region with ‘classical’ resolvases [12]. However, they have an extended and divergent C-terminal region. Members of this family of recombinases are required for insertion and excision of phage genomes, excision of DNA from genes involved in bacterial developmental processes, excision and insertion of mobiliseable transposons (covered in detail in the review by Adams et al. in this volume), and insertion and excision of a conjugative transposon Tn*5397* [13].

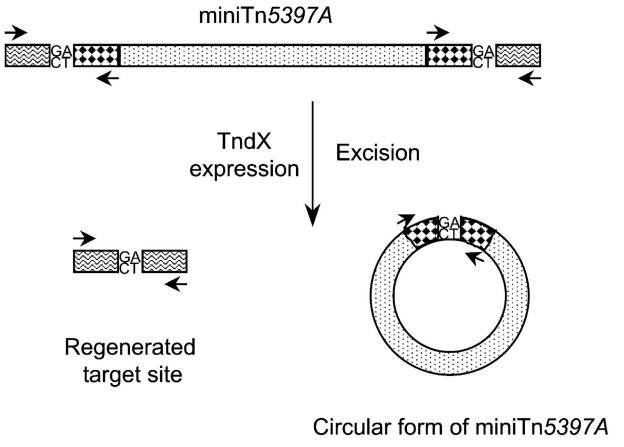

Figure 4. Schematic diagram showing the minitransposon (miniTn*5397A*) used in the excision assay for Tn*5397*. The ends of the transposon are represented by the diamond boxes. The flanking DNA is represented by boxes with wavy lines. The GA dinucleotides that flank the transposon are also shown. The central region of the minitransposon is represented by a dotted box. To construct the minitransposon the central region of Tn*5397* was replaced by a 1.7-kb chloramphenicol resistance gene which acted as a spacer between the left and right ends of the element. On expression of TndX in trans, the element excises to form a circular molecule which contains a GA dinucleotide at the joint between the left and right ends of the element. The original target site is also regenerated. The products of the reaction were detected by polymerase chain reaction (PCR) as previously described [17]. The binding sites of primers used in the PCR reactions are shown by arrows.

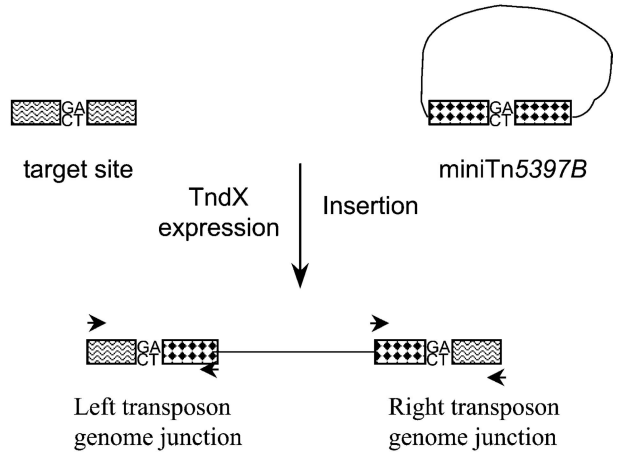

Figure 5. Schematic diagram showing the insertion assay. For this assay a minitransposon (miniTn*5397B*) was constructed. This essentially mimics the circular form of the transposon with the left and right ends of the element joined via the GA dinucleotide. This joint region was cloned into an *E. coli* vector (represented by the thin line). The ends of the transposon and the flanking DNA are represented as in figure 4. On expression of TndX, left and right transposon target site junctions are generated. These products were detected by PCR as previously described [17]. The arrows represent the binding sites of the PCR primers used in these assays.

Excision and insertion of Tn*5397* is mediated the large serine recombinase TndX

Tn*5397* is a conjugative transposon that was originally identified in the pathogen *Clostridium difficile* [14, 15]. Its complete DNA sequence has been obtained, showing that the element is very closely related to Tn*916* in the central region, but that the ends of the two elements are completely different (fig. 1) [16]. In Tn*5397*, the *tndX* gene, predicted to encode a protein of the class V serine recombinase family, is present instead of the *int* and *xis* genes of Tn*916* [13, 16].

Copies of Tn*5397* inserted in the *C. difficile* and *Bacillus subtilis* genome are flanked by a GA dinucleotide. The Tn*5397* target sites also contain a central GA dinucleotide, as does the circular form of the transposon at the joint between the left and right ends (fig. 4) [13]. After excision of Tn*5397* from the *C. difficile* genome the original target site is completely regenerated [13].

The *tndX* gene is required for conjugative transposition of Tn*5397*. A mutant transposon in which this gene is deleted is incapable of conjugative transfer or production of the circular form of the element [13]. TndX provided in trans will catalyse insertion and excision of miniTn*5397* derivatives; details of these assays are shown in figs 4 and 5. Furthermore, the transposition products are the same as those observed in wild-type Tn*5397* in *C. difficile* [17], proving that TndX is the only Tn*5397*-encoded protein required for transposition.

That TndX mediated excision and insertion by a similar mechanism to the resolvase enzymes was shown by the fact that point mutations in the conserved amino acids required for resolvase action completely abolished both excision and insertion [17]. The above data had led us to propose the following model for TndX-mediated insertion and excision of Tn*5397*: TndX mediates the excision of Tn*5397* by introducing 2-bp staggered cuts at the 3′ ends of the directly repeated GA dinucleotides at each end of the transposon. Strand exchange occurs, resulting in excision of the transposon as a circular molecule and regeneration of the original target site. A single strand of the circular form can then be transferred to a new host cell by conjugation. In the recipient cell a new strand is synthesised, and the double-stranded element is subsequently inserted into the recipient genome. Insertion is thought to be the reverse of excision.

Further work is required to understand the detailed molecular events involved in TndX recognition and binding to its target sites. Also, we do not yet know whether there is any control over directionality of recombination (i.e. integration verses excision).

Regulation of conjugative transposition of Tn*5397*

Tn*5397* contains genes that are very closely related to the key regulatory regions of Tn*916*, i.e. *orf7, orf8* and *orf9*, although there are some minor variations. However, the putative regulatory region upstream of the *tet*(M) gene is different, and there are no obvious promoters just upstream of *tndX* that would be equivalent to P*xis* or P*int*. Therefore, although they have elements in common, regulation of *tndX* in Tn*5397* is likely to be appreciably different from regulation of *int* and *xis* in Tn*916*.

So far, Tn*5397* is the only conjugative transposon proven to be dependent on a serine recombinase for mobility. However, database searches of bacterial genomes has shown the presence of large serine recombinases within putative elements that could be conjugative transposons. It would be instructive to examine these elements further to determine whether they are active mobile elements dependent on the serine recombinases and to compare them functionally with Tn*5397*.

Conclusions

Conjugative transposons are defined as genetic elements that integrate into the bacterial chromosome and transfer from a donor to a recipient bacterium. Crucial to this is their ability to excise and integrate from bacterial DNA. All conjugative transposons investigated so far use conservative site-specific recombinases for this purpose. The majority use enzymes of the tyrosine recombinase family of integrases, and there is one proven example of a serine recombinase being used.

The enzymes of the tyrosine family of integrases all seem to require accessory factors, at least for excision, whereas the serine recombinase TndX can mediate both insertion and excision. A feature of the recombinases of the conjugative transposons is their ability to operate in many different bacterial hosts and the fact that they have a minimal requirement for specific host factors. This almost certainly contributes to their exceptionally broad host range, accounting for the spread of the antibiotic resistance genes that these elements frequently carry.

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