Microbiology Spectrum
American Society for Microbiology Press

The Tn*3*-family of Replicative Transposons

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ABSTRACT Transposons of the Tn*3* family form a widespread and remarkably homogeneous group of bacterial transposable elements in terms of transposition functions and an extremely versatile system for mediating gene reassortment and genomic plasticity owing to their modular organization. They have made major contributions to antimicrobial drug resistance dissemination or to endowing environmental bacteria with novel catabolic capacities. Here, we discuss the dynamic aspects inherent to the diversity and mosaic structure of Tn*3*-family transposons and their derivatives. We also provide an overview of current knowledge of the replicative transposition mechanism of the family, emphasizing most recent work aimed at understanding this mechanism at the biochemical level. Previous and recent data are put in perspective with those obtained for other transposable elements to build up a tentative model linking the activities of the Tn*3*-family transposase protein with the cellular process of DNA replication, suggesting new lines for further investigation. Finally, we summarize our current view of the DNA site-specific recombination mechanisms responsible for converting replicative transposition intermediates into final products, comparing paradigm systems using a serine recombinase with more recently characterized systems that use a tyrosine recombinase.

INTRODUCTION

The ampicillin-resistance transposon Tn*3* is the archetype ("Tn*3*" being synonymous with "Tn*1*" or "Tn*2*"; (1)) of a large and widespread family of transposons with representatives in nearly all bacterial phyla including proteobacteria, firmicutes, and cyanobacteria. Family members are modular platforms allowing assembly, diversification, and redistribution of an ever-growing arsenal of antimicrobial resistance genes, thereby contributing along with other mobile genetic elements, to the emergence of multi-drug resistances at a rate that challenges the development of new treatments (2–4). They are also prevalent in horizontal transfer of large catabolic operons, allowing bacteria to metabolize various families of compounds, including industrial xenobiotic pollutants (5, 6).

A distinguishing feature contributing to the proliferation and evolutionary success of Tn*3*-family transposons is their replicative mode of transposition (7, 8) (Fig. 1). They transpose using a "copy-in" (or "paste-and-copy") mechanism in which replication of the transposon occurs during integration into the target. Intermolecular transposition generates a cointegrate intermediate, in which the donor and target molecules are fused by directly repeated transposon copies (Fig. 1). Cointegrate formation requires both the transposon-encoded transposase (TnpA), to cut and rejoin the transposon ends with the target DNA, and the cell replication machinery, to copy the complementary strands of the transposon.

Received: 31 October 2014, **Accepted:** 3 November 2014, **Published:** 23 July 2015

Editors: Mick Chandler, Université Paul Sabatier, Toulouse, France, and Nancy Craig, Johns Hopkins University, Baltimore, MD

Citation: Nicolas E, Lambin M, Dandoy D, Galloy C, Nguyen N, Oger CA, Hallet B. 2014. The Tn*3*-family of replicative transposons. *Microbiol Spectrum* 3(4):MDNA3-0060-2014. doi:10.1128/microbiolspec.MDNA3-0060-2014.

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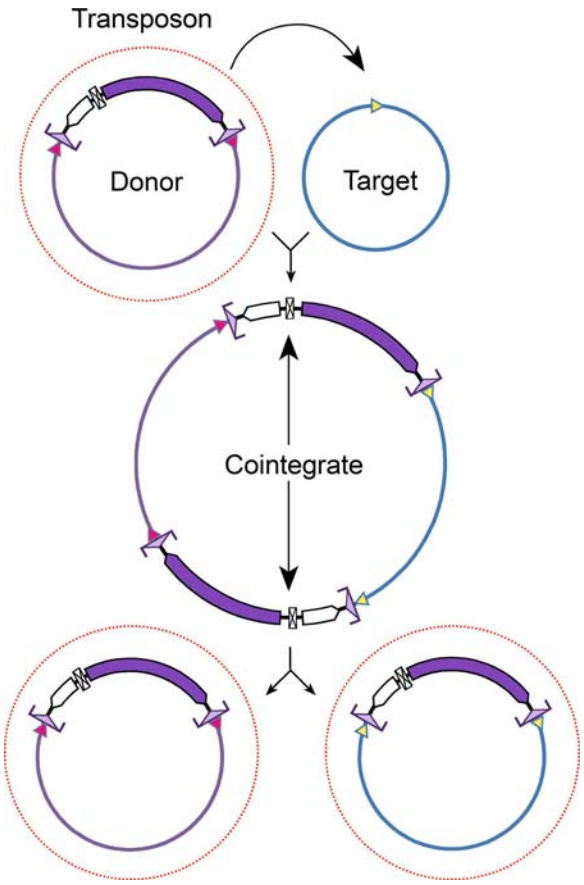


FIGURE 1 Overview of the replicative transposition cycle of Tn*3*-family transposons. Intermolecular transposition (curved arrow) from a donor (purple) to a target DNA molecule (blue) generates a cointegrate in which both molecules are fused together by directly repeated copies of the transposon. This step requires the transposase and the host replication machinery. The cointegrate is resolved by resolvase-mediated site-specific recombination (double arrow) between the duplicated copies of the transposon resolution site (boxed cross). Bracketed triangles are the terminal inverted repeats (IRs) of the transposon. The transposase and resolvase genes are represented by a purple and a white arrow, respectively. Small triangles show the short (usually 5-bp) direct repeats (DRs) that are generated upon insertion into the target. The red stippled circle indicates that a molecule that contains a copy of the transposon is immunized against further insertions due to target immunity. doi:10.1128/microbiolspec.MDNA3-0060-2014.f1

The transposase is also involved in a process termed target immunity whereby the transposon avoids inserting more than once into the same DNA molecule (7). This process is specific to each member of the family and is thought to act over large distances (up to several dozens of kilobases) within the genome.

Tn*3*-family transposons generally encode a DNA site-specific recombinase, or "resolvase", whose function is to resolve the cointegrate intermediate by catalyzing a reciprocal recombination reaction between the newly synthesized copies of the element (Fig. 1). Recombination takes place at a specific site, the "resolution site" (*res*) or "internal recombination site" (IRS) located inside the transposon (7). The reaction completes the transposition cycle, restoring the original donor molecule and producing a target molecule with a transposon copy (Fig. 1). The resolvase of most characterized Tn*3*-family transposons is a member of the serine recombinase (S-recombinase) family, but in a few cases it is a member of the tyrosine recombinase (Y-recombinase) family (7, 9).

Although cointegrate resolution has been the subject of intense biochemical, topological, and structural studies over the past decades, much less is known regarding the transposase-catalyzed reactions in both the transposition and target immunity processes. After an overview of the biological diversity and significance of Tn*3*-family transposons, this chapter will provide an update of the mechanistic aspects of the transposition cycle with a special focus on the initial steps of the reaction.

MODULAR AND DYNAMIC STRUCTURE OF TN*3*-FAMILY TRANSPOSONS

Mobile genetic elements can be described as a juxtaposition of functional modules, which together provide each element with its own specificities (10). Members of the Tn*3* transposon family include three types of module (Fig. 2): the core transposition module, which is the mobility signature of the family; the cointegrate resolution module, which optimizes the transposition pathway by reducing the risk of generating aberrant replicon fusions and making the transposon less dependent on the host recombination functions; and various sets of cargo genes and operons that were assimilated, presumably because they proved to be useful for their host under certain conditions.

The transposition module: the ID of the family

Autonomous Tn*3*-family members carry a transposition core module comprising the transposase gene (*tnpA*) and typical ~38-bp inverted repeats (IRs) at the transposon ends (Fig. 2). TnpA proteins are unusually large (from ~950 to ~1020 amino acids [aa]) compared to other transposases. A BLAST search performed with any Tn*3*-family transposase identifies only other members of the family. However, phylogenetic analysis of 924 full-length transposases from the Pfam database (http://pfam.xfam.org/) reveals five protein clusters sharing less than ~30% sequence identity (Fig. 3). One large cluster comprising

595 sequences can be further divided into three subgroups exemplified by the Tn*4430*, Tn*5393*, and Tn*21* transposases (Fig. 3). An alignment of 21 active transposases from the different clusters and separate bacterial phyla showed that only 15 residues, including the "DDE" catalytic triad, are perfectly conserved (11) (see also *The transposase* section, below).

Remarkably, this phylogeny does not faithfully superimpose on that of the bacterial species from which the transposons were originally isolated, underlining the important level of horizontal transfer associated with the family. In contrast, there is a good correlation between the transposase phylogeny and the ~38-bp terminal transposon IRs, suggesting that both transposition module partners have coevolved to maintain specific and functional interactions (see *Interaction with the transposon ends* section, below).

Transposition module-only Tn*3*-family elements

Certain representatives from phylogenetically separate Tn*3*-family subgroups are distinctive because they contain only the transposition module, an organization that relates these elements to insertion sequences (ISs) (12) (see also the chapter by P. Siguier *et al.* in this volume) (Fig. 2). Cointegrate intermediates generated by these 'IS-like' Tn*3*-family members are thought to be resolved by RecA-dependent homologous recombination. The lack of a transposon-encoded resolution system may have facilitated formation of stable composite transposons that are typical of IS families, in which two copies of the element became associated with other genetic determinants to mediate their mobility as a single entity (12) (Fig. 2).

One of these, IS*1071*, was initially identified for its contribution to the 17-kb chlorobenzoate-catabolic *Comamonas testosteroni* (formerly *Alcaligenes* sp.) transposon, Tn*5271* (13). Similar associations were subsequently found between intact or truncated IS*1071* copies and different catabolic gene clusters from a variety of bacterial isolates (5, 14). Another "transposition module-only" Tn*3*-family transposon, IS*3000*, was identified adjacent to the extended-spectrum β-lactamase CTX-M-9 gene from enterobacteria, and more recently, on the multi-resistance *Klebsiella pneumoniae* plasmid pKp11-42 (15, 16).

Because of its minimal organization, IS*1071* was initially proposed as the archetype of an ancestral element from which the other Tn*3*-family transposons evolved by acquisition of a cointegrate resolution system (7, 13). However, the TnpA proteins of IS*1071* and IS*3000* belong to separate clusters within the Tn*3*-transposase family (Fig. 3), suggesting that these minimal structures have evolved separately.

The cointegrate resolution modules

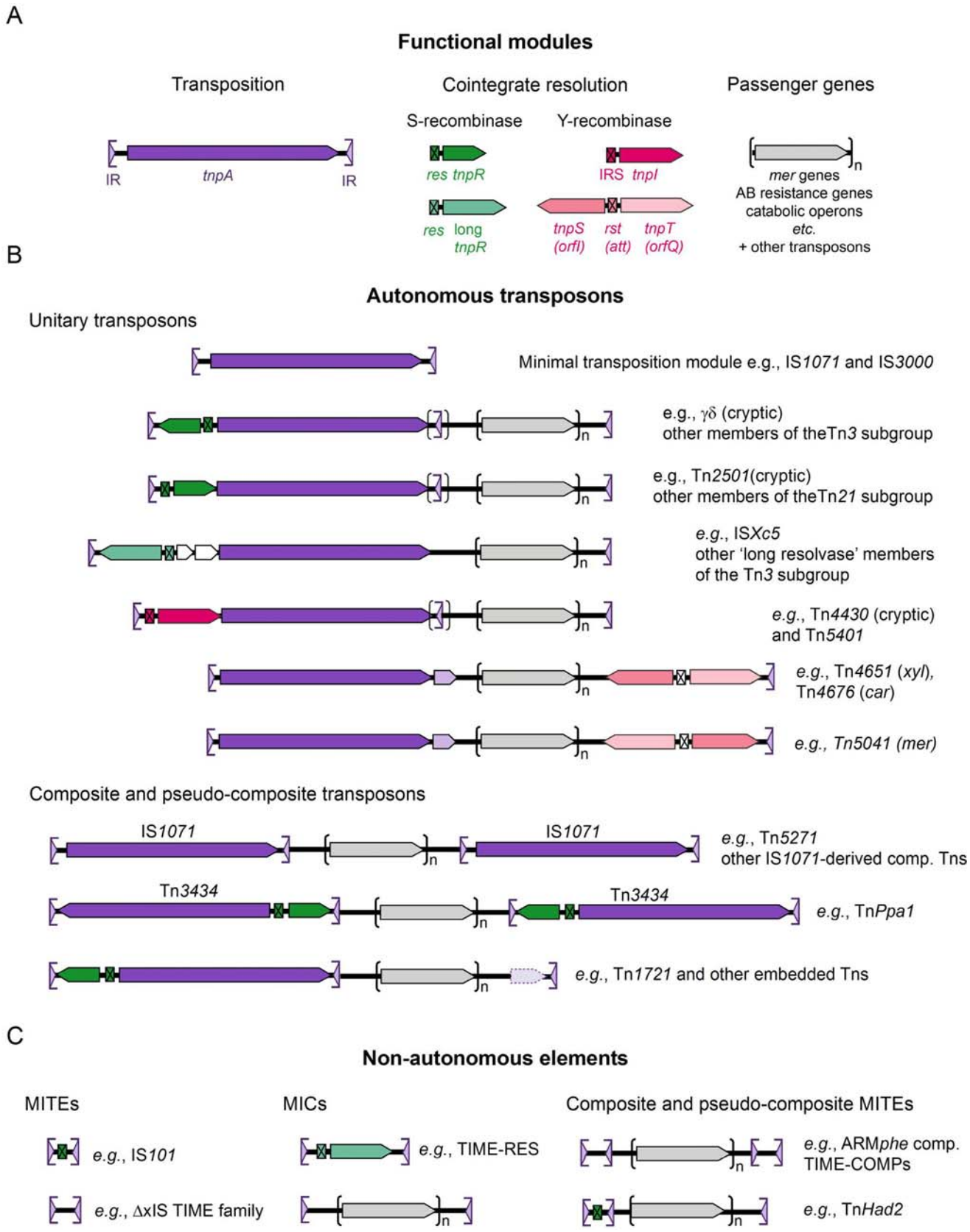
S-recombinases and Y-recombinases cleave and reseal DNA molecules at specific sequences using a serine or a tyrosine nucleophile, respectively. Both recombinase families are involved in a broad range of biological processes such as integration and excision of temperate bacteriophages, movement of different classes of mobile genetic elements and control of gene expression through programmed DNA rearrangements (17) (see also the section on "Conservative site-specific recombination" of this volume). In all of these types of biological process, there are clear cases where members of either family have been recruited to carry out similar functions. Cointegrate resolution systems of both recombinase families are for example closely related to those found on circular plasmids and chromosomes, the function of which is to reduce multimeric replicon forms and permit their segregation at the time of cell division (9) (Fig. 4 and 5).

Recombinases of both families mediate recombination between short (~28-bp to 30-bp) DNA sequences, the "core" sites, which typically contain inversely oriented recognition motifs separated by a central 2-bp (S-recombinases) or 6-bp to 8-bp (Y-recombinases) region (Fig. 6 and 7). The minimal core is usually insufficient to support efficient recombination. Recombination sites often contain extra DNA sequences to which additional recombinase subunits and/or accessory proteins bind to control the outcome of recombination. For transposon resolution systems, this is important to efficiently resolve cointegrate intermediates while avoiding detrimental DNA rearrangements (see *Convergent mechanisms to control the selectivity of recombination* section, below).

Cointegrate resolution modules using an S-recombinase

The S-resolvases

Tn*3*-family S-resolvases are typically small (~180 to 210 aa) comprising a relatively well conserved ~120-aa N-terminal catalytic domain connected to a short ~65-aa C-terminal DNA-binding domain (Fig. 6). However, the resolvases of some transposons (e.g., IS*Xc5* and Tn*5044* from *Xanthomonas campestris* or Tn*5063* and Tn*5046* from *Pseudomonas* sp.) are larger (~310 aa) due to a ~110-aa C-terminal extension (18–21) (Fig. 6). The role of this extension is not known. Its deletion abolished Tn*5044* resolvase recombination *in vivo* (18)



but had no effect on ISXc*5* resolvase activity (22). As previously observed (7), the S-resolvase phylogeny does not perfectly match that of the transposases, suggesting that cointegrate resolution modules were independently acquired or exchanged between transposons of the different subgroups after the divergence of their transposition module (Fig. 4).

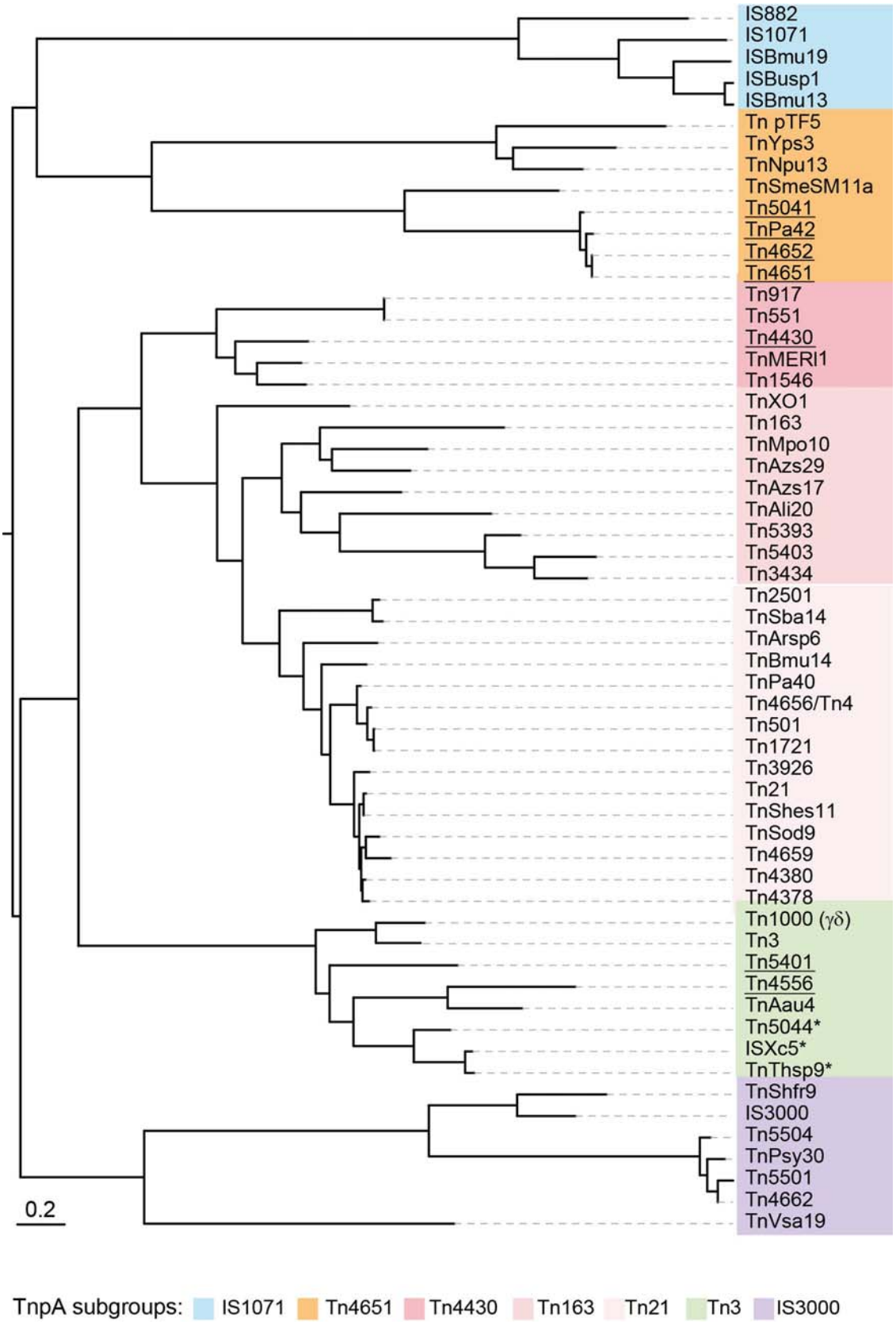
Variations in S-resolvase *res* site organization

The transposon resolution site, *res*, is generally located immediately upstream of the resolvase gene (Fig. 6). The best-characterized Tn*3*-family *res* sites, those of Tn*3* and γδ (also called Tn*1000*), are ~120 bp long and contain three subsites (I to III). Each subsite is composed of inversely oriented 12-bp resolvase recognition motifs (Fig. 6). Site I is the recombination core site (or crossover site) at which strand exchange occurs; sites II and III are regulatory elements required for assembly of a topologically defined synaptic complex to control recombination directionality (see *Convergent mechanisms to control the selectivity of recombination* section, below).

The three-subsite organization of *res* is conserved in most Tn*3*-family transposons in spite of the differences in resolvase sequence specificity. There is some variation, however, in the spacer length between the core site I and the accessory sites II and III, ranging from four to seven integral helical turns (Fig. 6). Conservation of the helical phasing between sites I and II is important to properly align the core and regulatory resolvase subunits in the synaptic complex (23, 24).

A structurally different *res* site organization has been proposed for other transposons such as ISXc*5* in which the site II inverted repeats are replaced by direct repeats (7, 25). Tandem resolvase binding motifs were also identified in the putative *res* sites of several Tn*3*-family transposons from Gram-positive bacteria (e.g., Tn*1546* and TnXO*1*) (7, 25). These *res* sites also appear more compact with two resolvase binding sites instead of three (Fig. 6). This two-subsite organization was initially described for Sin and the β-recombinases of Gram-positive plasmids to which the Tn*1546* and TnXO*1* resolvases are only distant relatives (25, 26) (Fig. 4 and 6). Recombination mediated by these recombinases requires the host protein Hbsu (or any DNA bending protein like the nucleoid-associated protein HU, the integration host factor IHF, or high-mobility group of proteins [HMG] from eukaryotes) that binds the spacer segment between sites I and II, playing an architectural role in synaptic complex assembly (25, 27–31). Other serine recombinases involved in plasmid monomerization are more closely related to resolvases from cointegrate resolution systems (32) (Fig. 4). In some cases there is clear

FIGURE 2 The modular structure of Tn*3*-family transposons and derived elements. (A) Tn*3*-family transposons are constituted by the association of three classes of functional modules. The transposition module comprises the transposase gene (*tnpA*, purple arrow) and its associated ~38-bp inverted repeats (IRs, bracketed triangles). The cointegrate resolution module is made of a gene encoding a site-specific recombinase from the serine- (green) or tyrosine- (magenta) family and their cognate recombination site (boxed cross). Resolution modules working with a tyrosine recombinase of the TnpS/OrfI subgroup (pale magenta) contain an additional gene coding for an accessory recombination protein (TnpT/OrfQ). "Long *tnpR*" (pale green) refers to a resolvase gene that encodes a C-terminally extended member of the serine recombinase family. Passenger genes comprise a variety of phenotypic determinants and transposons that are specific to each element (gray arrows). (B) Autonomous transposons are characterized by a fully functional transposition module to mediate the mobility of the element *in cis*. The simplest elements (ISs) are solely constituted by the minimal transposition module. Most characterized transposons have a typical unitary structure in which the transposase gene and its associated modules are flanked by the IR ends. Unitary elements can associate to form composite transposons containing a pair of full-length elements flanking a specific genomic segment; or pseudo-composite transposons carrying an autonomous element on one side and an isolated IR end on the other side. (C) Nonautonomous elements are Tn*3*-family derivatives whose mobility requires a functional transposase to be provided in *Trans* by a related transposon. Miniature Inverted-repeats Transposable Elements (MITEs) are solely constituted by a pair of IR ends flanking a short DNA segment that sometime contains a cointegrate resolution site. Mobile Insertion Cassettes (MICs) are nonautonomous unitary elements carrying one or more passenger genes between the ends. MITEs can also associate with passenger genes and isolated ends to form composite and pseudo-composite mobilized structures. See the text for details and the indicated examples. doi:10.1128/microbiolspec.MDNA3-0060-2014.f2



evidence that they were derived from transposons during recent evolution (25, 32).

Finally, related resolution systems are also found in a distinct class of transposable elements called "Mu-like transposons" for their resemblance to bacteriophage Mu. These include β-lactam resistance transposon Tn*552* from *Staphylococcus* sp. and transposons of the widespread Tn*5053*/Tn*402* family (33–35) (Fig. 4 and 6). Like Tn*3*-family transposons, these move by replicative transposition and generate cointegrates. The Tn*552* BinL resolvase is a close homolog of the plasmid pI9789 BinR recombinase, whereas the Tn*5053*/Tn*402* TniR recombinase is closer to the resolvase of classical Tn*3*-family members like Tn*163* and Tn*5393* (Fig. 4). TniR and BinL are also distant relatives of the C-terminally extended resolvases (e.g., those of ISXc*5* and Tn*5044*; Fig. 4) and the organization of the *resL* site of Tn*552* was proposed to resemble the resolution site of ISXc*5* (7, 25) (Fig. 6).

Cointegrate resolution modules using a Y-recombinase

Phylogenetically distinct Y-resolvase subgroups

Y-recombinases fall into well-separated subgroups based on sequence similarities, which correlate with different recombination site organization (Fig. 5 and 7). As for the S-resolvases, recombinases of the different subgroups are phylogenetically related to other Y-recombinases that play a role in stabilizing circular replicons (Fig. 5).

Recombinases of one group, exemplified by TnpI of *Bacillus thuringiensis* transposons Tn*4430* (285 aa) and Tn*5401* (306 aa) (36, 37) are distant homologs of the chromosomally encoded multifunctional XerCD recombinases whose primary function is to resolve chromosome dimers arising from homologous recombination before their segregation into daughter cells (38–40) (see also the chapter by C. Midonet and F.-X. Barre in this volume).

The TnpS/OrfI recombinases (323 aa and 351 aa, respectively) encoded by the *Pseudomonas* sp. Tn*4651*/Tn*5041* transposon subfamily (41–43) represent a second protein group more closely related to bacteriophage P1 Cre protein and to recombinases of a variety of large *Rhizobiacae* plasmids (9) (Fig. 5). Interestingly, TnpS-mediated or OrfI-mediated cointegrate resolution requires the product of a divergently oriented gene (TnpT or OrfQ) that shows no homology to any other characterized protein (41, 44).

Another putative Y-resolvase type identified in Tn*4556* from *Streptomyces fradiae* is more closely related to the ResD resolvase of *Escherichia coli* F factor than to the two other groups (7, 45) (Fig. 5). Finally, the defective *Pseudomonas putida* transposon Tn*4655* carries a unique site-specific recombination module composed of a recombination site (*attI*) and a Y-recombinase (TnpI) substantially larger (415 aa) than the other resolvases of the family (46). This system was proposed to have been initially involved in gene exchange between different replicons before incorporation as a bona fide cointegrate resolution system (46).

Variations in Y-resolvase *res* site organization

The IRS of Tn*4430* (116 bp) is located immediately upstream of *tnpI* (Fig. 7). It is composed of four TnpI-binding motifs with a common 9-bp sequence. Two 16-bp inverted motifs (IR1 and IR2) form the IRS core recombination site, whereas the adjacent 14-bp direct repeats (DR1 and DR2) are dispensable but required to control selectivity and directionality (36, 47) (Fig. 7) (see also *Variation on a theme: the TnpI/IRS recombination complex of Tn4430* section, below). The IRS of Tn*5401* exhibits a similar arrangement of TnpI binding motifs suggesting a similar mechanism to control cointegrate resolution (37, 48) (Fig. 7).

A completely different organization has been proposed for the Tn*4651*/Tn*5041* subfamily resolution site (designated "*rst*" for the resolution site associated with TnpS and TnpT) (41, 44). Deletion analysis located the minimal requirement for full *rst* activity to a 136-bp sequence between *tnpS* and *tnpT* (Fig. 7). DNA strand exchange takes place at a 33-bp inverted repeat (IRL-IRR), dividing *rst* into two arms (44). A similar 34-bp cross-over site was previously proposed for Tn*5041* (42). The accessory protein, TnpT, binds to another conserved 20-bp inverted repeat element (IR1–IR2) in the *tnpT*-proximal arm side of *rst* (44) (Fig. 7). However, it has yet to be determined how this protein collaborates with TnpS to mediate recombination.

FIGURE 3 Phylogenetic tree of the Tn*3*-family transposase proteins. The different clusters and subgroups identified within the family are boxed with different colors as indicated. Transposons that contain a cointegrate resolution module working with a tyrosine recombinase are underlined. Transposons that encode a "long", C-terminally extended resolvase of the serine-recombinase family are marked with an asterisk. The length of the branches is proportional to the average number of substitutions per residue. doi:10.1128/microbiolspec.MDNA3-0060-2014.f3

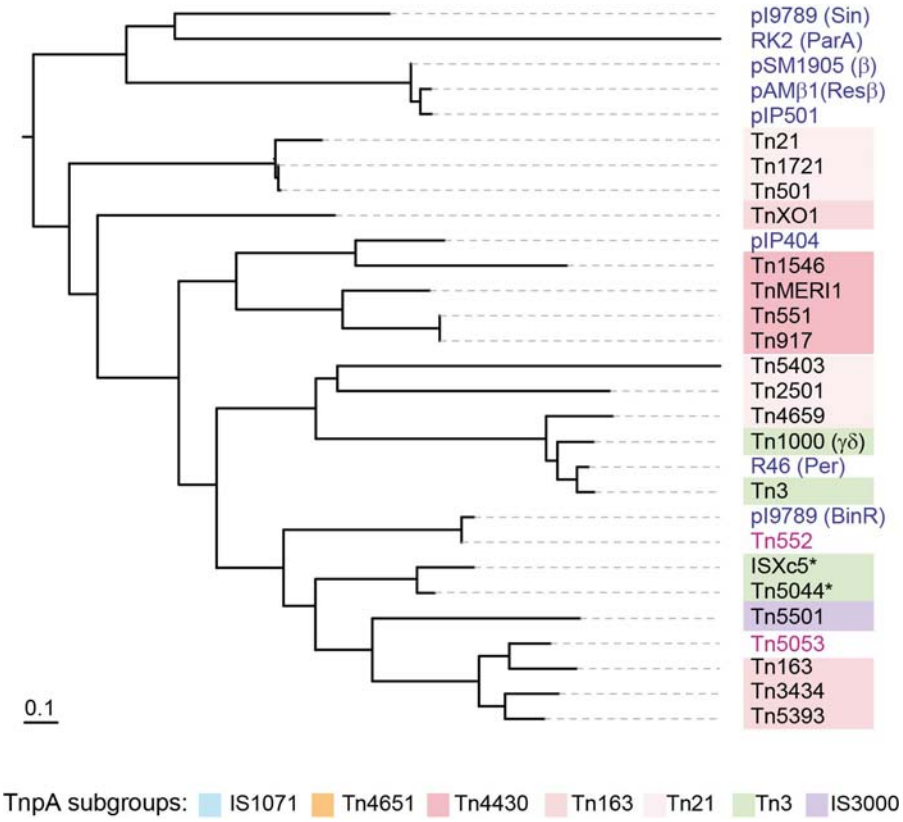


FIGURE 4 Phylogenetic tree of transposon-encoded and plasmid-encoded resolvases of the S-recombinase family. Transposons belonging to different subgroups are boxed with different colors as in Fig. 3. Transposons encoding a "long", C-terminally extended resolvase are marked by an asterisk. Plasmids are highlighted in blue with the name of the corresponding recombinase (when assigned) in brackets. "Mu-like" transposons are highlighted in magenta. The length of the branches is proportional to the average number of substitutions per residue. doi:10.1128/microbiolspec.MDNA3-0060-2014.f4

Cryptic transposons: conduction and formation of compound structures

A number of transposons belonging to different Tn*3*-family subgroups are phenotypically "cryptic" and encode no functions other than those associated with transposition and cointegrate resolution (Fig. 2). Replicative transposons of these elements can promote gene exchange by forming transient cointegrates between a conjugative plasmid and non-self-transmissible genetic material in a process known as "conduction" (49). This process led to the identification and initial characterization of several Tn*3*-family members (49–51).

The process may potentially impact public health. For example, the cryptic transposon Tn*4430* from the large self-transmissible *B. thuringiensis* pXO12 plasmid mediates relatively high-frequency conduction of *Bacillus anthracis* virulence plasmids pXO1 and pXO2 among different *Bacillus cereus sensu lato* strains (52). More generally, Tn*4430* is thought to contribute, with other transposable elements, to diversification of the entomopathogenic specificity of *B. thuringiensis* serovars by promoting redistribution and subsequent recombination of the plasmid-encoded δ-endotoxin genes (53).

Cryptic transposons can form transpositionally active composite assemblies as exemplified by Tn*Ppa1*, a large genomic transposon from *Parococcus* spp (54, 55) (Fig. 2). Tn*Ppa1* is delineated by divergently oriented copies of the cryptic element Tn*3434* (3.7 kb). The mobilized Tn*Ppa1* core (37 kb) carries 34 open reading frames with a variety of predicted functions, such as housekeeping genes, different transporter families and transcriptional regulators (54, 55).

Passenger genes and operons

Tn*3*-family transposons are generally unitary, noncomposite structures (previously designated "class II" transposons) containing one or more passenger genes in addition to their transposition and cointegrate resolution modules (Fig. 2). The size of these elements varies from a few, to tens of kilobases depending on the number of genes they transport.

Antimicrobial passenger genes

Tn*3*-family members that confer resistance to antimicrobial compounds are found in different subgroups of the family. In the simplest cases, these elements encode one or more determinants active against a specific class

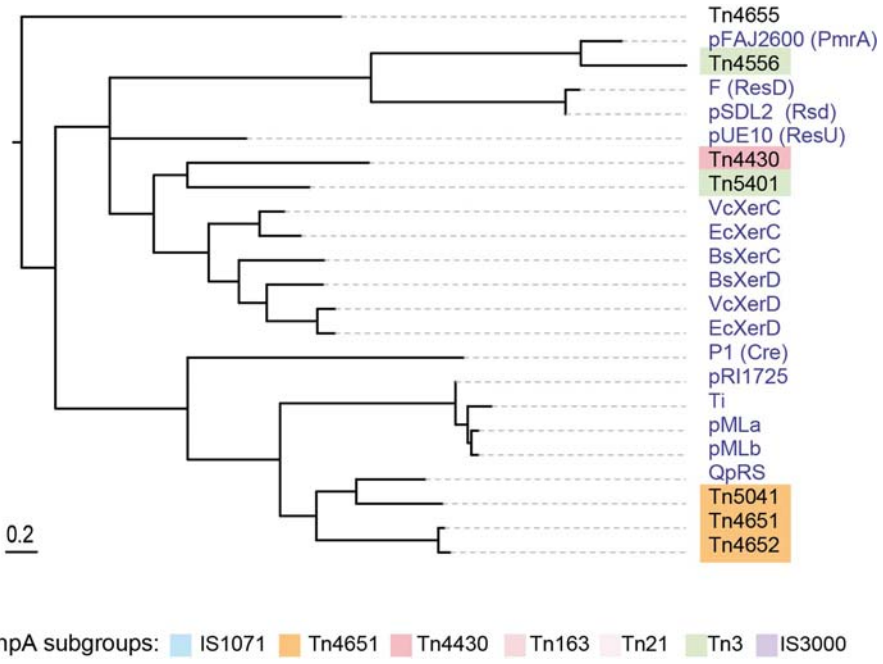


FIGURE 5 Phylogenetic tree of transposon-encoded and plasmid-encoded resolvases of the Y-recombinase family. Transposons belonging to different subgroups are boxed with different colors as in Fig. 3. Plasmids are highlighted in blue with the name of the corresponding recombinase (when assigned) in brackets. Representative and well-characterized XerC and D recombinases are from *Escherichia coli* (Ec), *Bacillus subtilis* (Bs) and *Vibrio cholerae* (Vc). The length of the branches is proportional to the average number of substitutions per residue. doi:10.1128/microbiolspec.MDNA3-0060-2014.f5

of compounds, sometimes associated with additional regulatory functions. Examples of these include the TEM-1 β-lactamase genes—Tn*3* and its derivatives (1), the inducible macrolide-lincosamide-streptogramin resistance gene *ermAM*—Tn*917* from *Enterococcus faecalis* (56), the aminoglycoside phosphotransefrase *strA* and *strB* genes—Tn*5393* (57) or the tetracycline efflux pump *tetA(A)* gene—Tn*1721* (58). Dissemination of vancomycin resistance by Tn*1546* (10.85 kb) from *Enterococcus faecium* involves a cluster of seven *van* genes. Five of these are required for the inducible synthesis of alternative peptidoglycan precursors that are refractory to the glycopeptide antibiotic (59).

Mercury-resistance transposons, which encode genetic determinants allowing detoxification of mercuric ions from the environment, are also found in different Tn*3* subfamilies. They are evolutionarily ancient and widely dispersed elements found in a variety of bacterial species and diverse environments (20, 60, 61). The mercury resistance operons (*mer*) comprise a variable number of genes depending on the element and its detoxification capabilities. In most cases, they include a metal-responsive regulator (*merR*), a transport system responsible for delivering mercuric ions into the cell (*merTP*), and a cytoplasmic mercuric reductase (*merA*) that converts toxic Hg^{2+} ions into less toxic reduced forms (61).

With the successive development of novel antibiotherapies, transposons initially active against only restricted ranges of compounds, some of which like mercury resistance transposons predated the antibiotic era, accumulated additional resistance genes, as the consequence of coselective pressure (see below). There are now many identified Tn*3* family representatives with different combinations of antibiotic resistance determinants, which contribute to the emergence and accelerated expansion of multi-resistant bacterial pathogens in both the clinical and community environments (3, 4, 61, 62).

Catabolic passenger genes

Tn*3*-family catabolic transposons are typically large (>40 kb) and enable the host cells to use xenobiotic compounds such as recalcitrant aromatic hydrocarbons (e.g., toluene, xylenes, and naphthalene) as a sole source of carbon and energy. This generally relies on complex clusters of genes that often comprise an "upper" pathway operon that is responsible for the conversion of the initial substrate into oxidized intermediates, a "meta" operon that is required to degrade the resulting intermediates into central metabolites, and additional regulatory genes that are involved in the transcriptional activation of the whole pathway (5, 6).

Pathogenicity and other passenger genes

Other functions associated with Tn*3*-family transposons include virulence factors from both animal and plant pathogens. For example, the *B. anthracis* virulence plasmid pXO1 transposon TnX*O1* (8.7 kb) carries the *ger*XBAC operon responsible for spore germination

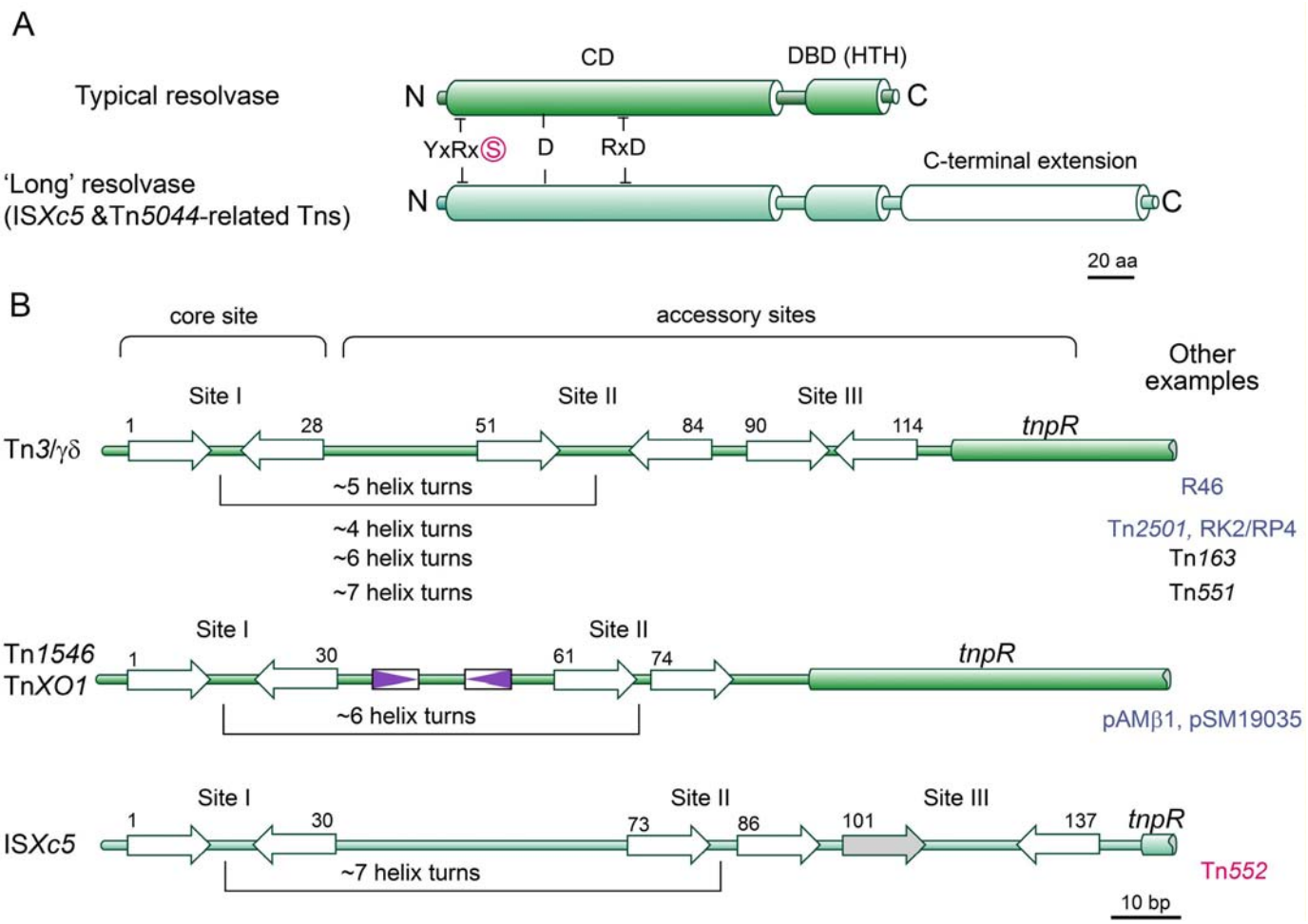


FIGURE 6 Cointegrate resolution modules working with a resolvase of the S-recombinase family. (A) Serine-resolvases are typically small proteins containing an N-terminal catalytic domain (CD) linked to a short helix-turn-helix (HTH) C-terminal DNA-binding domain (DBD). Resolvases of the "long" resolvase subgroup have a ~100-amino acid extension (white cylinder) at the C-terminus. Position of important catalytic residues (248) is indicated, with the active site serine (circled) highlighted in magenta. (B) Organization of the resolution sites *res*. Open arrows are the 12-bp resolvase binding motifs. Shaded arrows are for motifs with a poorer match to the consensus. Coordinates (in bp) of the first position of each motif are indicated above the recombination sites. Boxed triangles show the position of the putative Hbsu binding sites in the *res* site of Tn*1546* and Tn*XO1*. Organization of Tn*1546*, Tn*XO1* and IS*Xc5* res sites is as proposed by Rowland *et. al.* (25). Adapted from figure 6, p. 152 of reference (9). doi:10.1128/microbiolspec.MDNA3-0060-2014.f6

during infection (63, 64) and the *Pseudomonas syringae* Tn*hopX1* promotes dispersal of HopX1, a conserved effector protein present in most plant pathogens with type III secretion systems (65). Recent bioinformatics analyses reveal that the contribution of Tn*3*-related elements to plant virulence is more general, being involved in the spread of different classes of secreted type III effectors in the Xanthomonads (A. Varani and M. Chandler, personal communication). A few transposons from separate subgroups encode post-segregational killing toxin–antitoxin systems (59, 66, 67). This presumably helps to stabilize the transposon in a bacterial population.

The dynamics of Tn*3*-family transposon modular organization: module capture and reshuffling

It is often difficult to predict the exact mechanisms by which the Tn*3* family building blocks have been

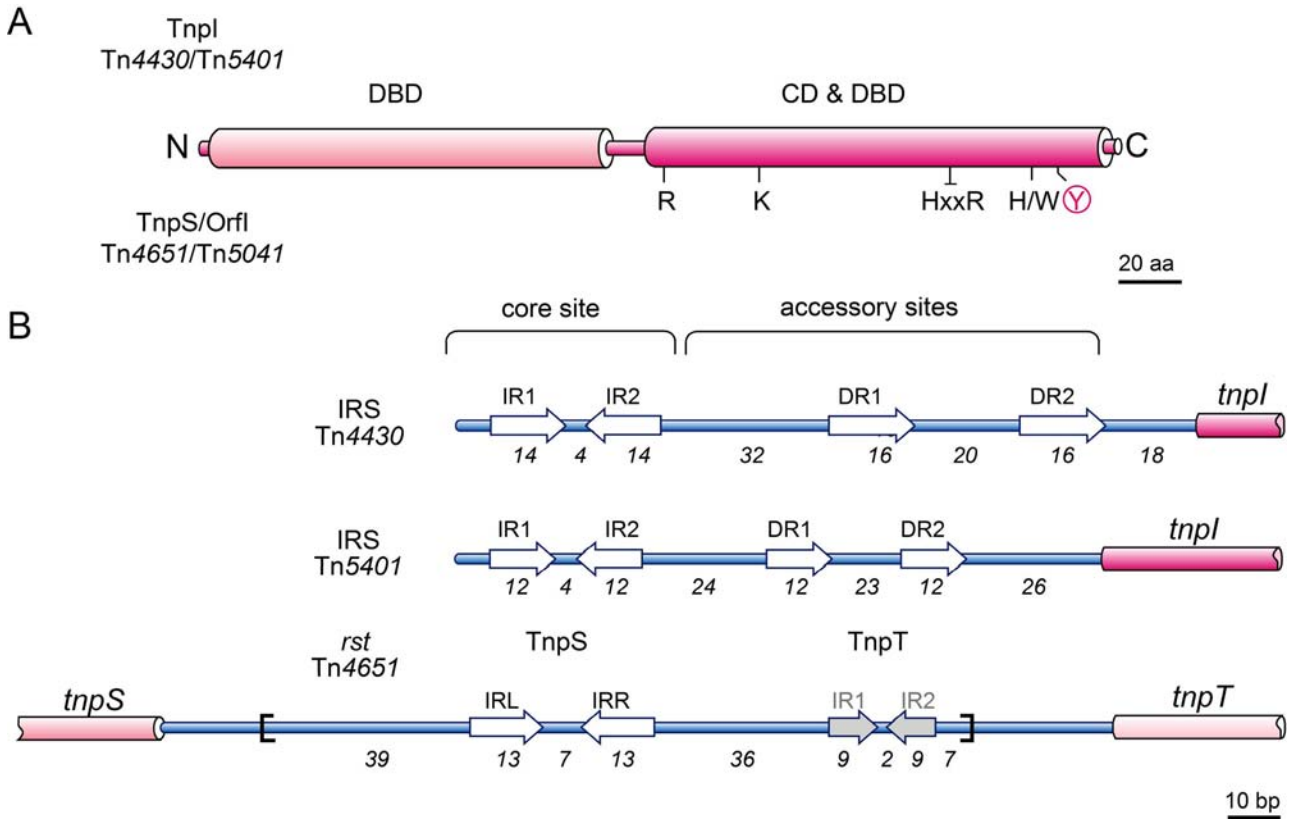


FIGURE 7 Cointegrate resolution modules working with a resolvase of the Y-recombinase family. (A) Typical two-domain structure of a tyrosine recombinase. The catalytic domain is in the C-terminal part of the protein. Positions of the conserved catalytic residues ($RKHR^{H}/_{W}Y$) are indicated, with the active site tyrosine (circled) highlighted in magenta. Both the N- and C-terminal domains contain the DNA-binding determinants of the protein (DBD). (B) Organization of the transposon recombination sites. Open arrows indicate the recombinase binding motifs. Shaded arrows in the *rst* site of Tn*4651* are the putative DNA recognition motifs for the auxiliary protein TnpT. Numbers indicate the length and the spacing between each motif (in bp) in the recombination site. Brackets in the *rst* site show the extent of the functional recombination site as determined by deletion analysis (44).
doi:10.1128/microbiolspec.MDNA3-0060-2014.f7

assembled to generate their specific modular structures. Although each element has its own history, several general, nonexclusive scenarios can be drawn based on certain observations.

Creating and sharing new transposon ends

Acquisition of new phenotypic traits may occur when the proximal end of a transposon inserted adjacent to a selectable marker gene is inactivated and a more distant "surrogate" IR sequence is recruited. This was suggested to occur during acquisition of the carbapenemase bla_{KPC} gene by the transposon Tn*4401* (68) and presumably in other single-trait Tn*3*-family transposons. In Tn*3*-related elements, a degenerate "ancestral" IR sequence is found immediately upstream of the bla_{TEM1} passenger gene (our unpublished observation); whereas in Tn*4401* the supernumerary internal IR end was inactivated by insertion of IS*Kpn7*, which also reinforced expression of the bla_{KPC} gene (68–70).

Unitary transposons may also be derived from composite transposons by inactivation of one of the two flanking transposon copies. This is the case for the tetracycline resistance transposon Tn*1721*, in which one element of the initial composite structure is inactivated by deletion leaving the other element (Tn*1722*) intact and capable of autonomous transposition (58, 71) (see also Fig. 2). In addition, Tn*1721*, like several other Tn*3*-family members, mediates one-ended transposition, a poorly understood transposition reaction catalyzed by the transposase at a single IR end that generates recombinant

products containing one terminus of the transposon and variable lengths of adjacent DNA (72–76). This can directly provide single-end derivatives for the construction of new modular arrangements without requiring subsequent deletion events.

Combination of functional transposition modules is well illustrated by certain catabolic transposons that form large (up to ~90 kb) mosaic structures containing multiple mobile subentities (e.g., (46, 77–79)). This is viewed as an adaptive strategy allowing movement and reassortment of different combinations of catabolic functions within and between separate plasmids from environmental bacteria (6). A similar nested organization has been reported for other phenotypically distinct Tn*3*-family transposons such as the *Yersinia enterocolitica* lactose Tn*951* (80) or the mercury-resistance derivatives Tn*510* and Tn*511* (81). Some of these are transposition-defective and require an active copy of a related element to be mobilized in *trans* (46, 77, 80, 81).

Housing other transposable elements

There are specific recombination systems that facilitate acquisition of new passenger genes. A particularly striking example is the recruitment of mobile integrons, site-specific recombination systems that use a Y-recombinase family integrase (IntI) to promote the exchange of specific gene cassettes (82–84) (see also the chapter by J. A. Escudero *et al.* in this volume). This led to the rapid and incremental diversification of antibiotic resistance genes in the Tn*21*-related mercury-resistance transposons and other Tn*3*-family members (85, 86). The evolution of multidrug-resistant transposons has been proposed to retrace the recent history of antimicrobial treatments (for discussion, see references 2, 3, 87, and 88).

A proposed founding event at the onset of multidrug resistance evolution was the incorporation of a specific integron and its associated cassettes into a "Mu-like" Tn*5053*/Tn*402*-family transposon (33), generating a specific class of "mobile" integrons known as "class 1" or "Class 1 In/Tn" (82, 87). Although this may have produced different variants of the ancestral Class 1 In/Tn (89, 90), the key to introduction and spread of one particular type of Class 1 integron among bacterial pathogens was presumably the presence of a gene (*qacE*) conferring resistance to quaternary ammonium compounds, which were commonly used as biocides before the antibiotic era. Concurrently, bacteria also accumulated mercury-resistance transposons, notably because of industrial enrichment of Hg^{2+} in the environment and the use of mercury-containing disinfectants in clinical practices (2, 3, 88). Since Tn*402* family transposons preferentially integrate within *res* sites (91–93), coselection of both types of mobile element could have led to the formation of recombinant transposons with Class 1 In/Tns inserted within or close to the *res* site of mercury-resistant Tn*3*-family members. Integration of different variants occurred not only at separate positions within different Tn*21*-subgroup members but also in non-mercury-resistance transposons such as Tn*1721* (85, 86). With the introduction of successive classes of therapeutic antibiotics, the integrons of these different transposons subsequently acted as a platform for collecting and redistributing new resistance cassettes from the reservoir, thereby accelerating their accumulation and dissemination among both established and emerging pathogens.

Passenger genes can also be incorporated into Tn*3*-family members in the form of composite transposons (e.g., TnHad2, (77)) or through the promiscuous transposition activity of members of certain IS families (e.g., IS*Ecp1* [IS*1380* family] or IS*CRs* [IS*91* family]) able to transduce variable lengths of DNA adjacent to their integration site (2, 85, 94–97). IS*Ecp1* and IS*CR1* are for example responsible for incorporation of variants of extended-spectrum $bla_{CTX\text{-}M}$ genes into Tn*21*-related elements (e.g., Tn*1722*), or into class 1 integrons with which they are associated (98–102).

Mixing by recombination

Another major process driving Tn*3* family evolution is recombination between both related and unrelated transposons (85). This may occur by homologous recombination between common regions or by inter-transposon site-specific recombination at the *res* site (6, 20, 61, 85). Resolvase-mediated recombination between heterologous *res* sites has been inferred from sequence comparison (1, 20, 79, 103) or demonstrated experimentally (78, 104, 105). *Res* site recombination can occur both intra- or inter-molecularly and can also lead to inversions between adjacent transposons reflecting some promiscuity in the activity of certain S-resolvases, which are normally controlled by a mechanism of resolution selectivity (78, 105, 106) (see *Convergent mechanisms to control the selectivity of recombination* section, below). Similar activities were proposed for the TnpS/OrfI Y-recombinase of the Tn*4651*/Tn*5041* subgroup (43, 44) and for the atypical Tn*4656* TnpI/*attI* recombination system (46). Promiscuous recombination mediated by cointegrate resolution systems appears to be an ongoing process that constantly reshapes the Tn*3* family by exchanging functional module and passenger genes between its different members (6, 20, 61, 85).

MITEs and MICs: more tricks in the Tn*3*-family toolbox

Besides the minimal transposable modules described as IS elements, early studies identified an even more compact transposon derivative, IS*101*, for its ability to promote cointegrate formation between its parental plasmid, pSC101, and other replicons (107) (Fig. 2). This 209-bp element consists of a pair of IR ends flanking a typical *res* site. IS*101* activity required the γδ transposase and resolvase in *trans* (108). IS*101* is a typical "MITE" (Miniature Inverted repeat Transposable Element). These are short nonautonomous elements found in both eukaryotes and prokaryotes (109, 110), which can be mobilized by active copies of related transposons. Several Tn*3*-family MITEs have been identified (111) and have been called "TIMEs" (Tn*3* family-derived Inverted repeat Miniature Elements) (67) (Fig. 2). Tn*5563*-derived TIMEs form a rather dispersed family of elements in *Pseudomonas* sp. and several other proteobacteria (67).

Another derivative, "RES-MITE", was identified that retains the IR ends and a cointegrate resolution module, but lacks the transposase (67). This element is a particular case of another class of nonautonomous transposable elements, an "MIC" (Mobile Insertion Cassette), composed of transposon ends flanking one or more genes not directly involved in transposition (112, 113) (Fig. 2). MIC mobility, like that of MITEs, also requires a source of cognate transposase and there is evidence that structurally equivalent elements contribute to the Tn*3* family outfit of tools, for example by promoting the dissemination of plant virulence factors in *Xanthomonas* spp (A. Varani and M. Chandler, personal communication).

THE MOLECULAR MECHANISM OF TRANSPOSITION

Different models were initially proposed for the Tn*3* family "copy-in" replicative transposition mechanism and that of other cointegrate-forming elements such as bacteriophage Mu, Mu-like transposons of the Tn*552*/Tn*5053* groups, and the IS*6* family (12, 34, 35, 114–117). In one of these models, verified for bacteriophage Mu (114), the transposase introduces single-strand nicks at both 3′ ends of the element, and then joins the ends to staggered phosphates of the target DNA (strand transfer) to generate an intermediate (the "Shapiro intermediate") in which the transposon DNA strands are connected to both the donor and target molecules through three-way branched structures resembling replication forks (Fig. 8). Recruitment of host replication factors leads to transposon duplication and the formation of short direct repeats flanking both copies of the element in the cointegrate (Fig. 8).

Studies on Mu have provided a detailed molecular picture of the different stages in transposition complex assembly and progression (the transpososome), and how it is then disassembled to recruit the host replication machinery (117–119) (see also the chapter by R. M. Harshey in this volume). However, the relevance of this model to other replicative transposable elements, including the Tn*3*-family, is currently not known.

The transposase

During transposition, transposase must bind specifically to the transposon ends, interact with an appropriate target, and assemble a higher order nucleoprotein complex, the "transpososome", in which the transposition partners are brought together in a proper configuration to catalyze the DNA breakage and rejoining reactions. Additional accessory proteins often join the complex to control the outcome of transposition or to process reaction intermediates. As will be discussed below, Tn*3*-family transposases are also the specific determinant of target immunity, acting both as a "repulsive" signal to discard the transposon from immune targets and as a component of the transpososome that selectively responds to this signal.

A sequence alignment performed with a representative subset of transposases belonging to different Tn*3*-family subgroups revealed discrete regions of higher similarity (Fig. 9). Limited tryptic and α-chymotryptic proteolysis of the Tn*4430* transposase coupled to mass spectrometry showed that these conserved regions are included in separate structural domains (120).

The C-terminal catalytic domain

The most conserved transposase region overlaps with a ~27-kDa proteolytic fragment roughly corresponding to the C-terminal third of the protein and contains 9 of the 15 perfectly conserved residues including the three acidic residues that constitute the "DD-E" catalytic triad (7, 11) (Fig. 9). This motif is the signature of a superfamily of proteins, the DD-E/D polynucletotidyl transferases, that includes various nucleic-acid processing enzymes, as well as different transposase families and the retroviral integrases (121–123). These enzymes have a common "RNase H-like" fold of their catalytic core, in which the conserved triad provides the essential active site residues. Phosphodiester bond breakage and resealing reactions uses an Mg^{2+}-dependent transesterification mechanism with a water molecule or a 3′ OH group as a nucleophile (123, 124). The Tn*3*-family DD-E motif is

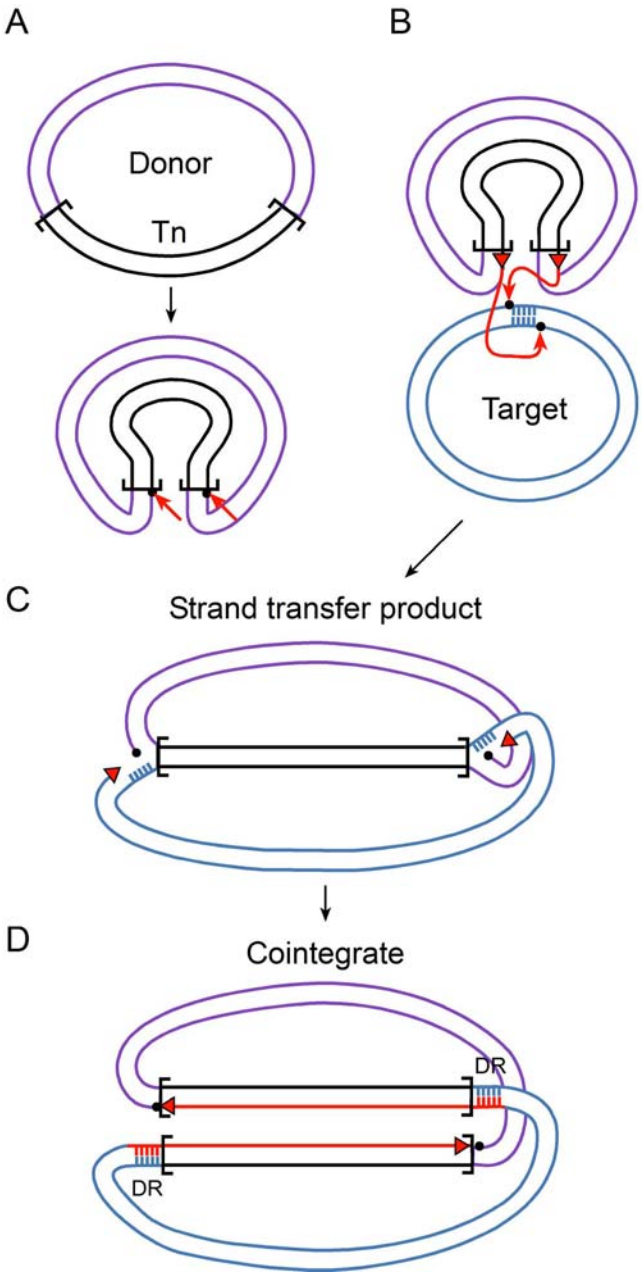


FIGURE 8 Model for copy-in replicative transposition. The diagram illustrates the case of intermolecular movement of a transposon (double black line delineated by brackets) between a donor molecule (purple) and a target molecule (blue). (A) Transposition starts when the transposase introduces specific single-strand nicks at both 3′ ends of the element, releasing 3′ OH groups in the donor (red triangles). (B) The transposon 3′ OH ends are then used as a nucleophile to attack separate phosphodiester bonds in both target DNA strands. (C) The reaction generates a strand transfer product (often called a "Shapiro intermediate") in which the transposon is linked to the target DNA through its 3′ ends, and to the donor DNA through its 5′ ends. The target phosphates (black dots) are usually staggered by five base pairs, which leaves 5-bp single-strand gaps at the junctions between the transposon and the target DNA in the strand transfer intermediate. (D) Replication initiates at the 3′ OH end(s) released by cleavage of the target to synthesize the complementary strands of the transposon (red line) and form the cointegrate. DNA synthesis also repairs the single-stranded gaps at the ends of the transposon generating directly repeated (DR) 5-bp target duplications that flank both copies of the element in the final cointegrate. doi:10.1128/microbiolspec.MDNA3-0060-2014.f8

surrounded by other highly conserved amino acid residues underlining the importance of this region. Mutation of the conserved Asp751 residue in the Tn*4430* transposase DD-E motif abolishes DNA cleavage and strand transfer *in vitro*, whereas the other activities (i.e., ability to bind and pair the transposon ends) are unaffected (Nicolas *et al.*, in preparation).

Secondary structure predictions suggest that the DD-E region exhibits the βββαβαβ-α topological arrangement typical of the RNase H fold, with the conserved catalytic residues occupying the expected positions within the predicted structure (120). This was confirmed by three-dimensional modeling using different protein threading tools, with the best fits being obtained with the catalytic core of HIV-1 integrase (Fig. 9) (M. Lambin and B. Hallet, unpublished data). However, the proposed Tn*3*-family RNAse H fold domain is predicted to contain an extra ~90-aa insertion between the last β strand of the central β sheet and the C-terminal α helix carrying the conserved Glu residue of the DD-E catalytic triad (120) (Fig. 9). This insert corresponds to one of the most conserved regions of the proteins, suggesting an important role in transposase activity. Insertion domains with different structures are found in an equivalent topological location of the Tn*5* and Hermes transposases where they are proposed to play a role in the formation of DNA hairpin intermediates for the excision of these elements (122, 125–127).

The N-terminal DNA-binding domain

Another highly conserved Tn*3*-family transposase segment lies at the N-terminus. Structural homology searches based on secondary structure predictions and fold recognition modeling revealed that this region has a high likelihood to form a bipartite DNA-binding domain analogous to that of the human centromeric protein CENP-B (128) (Fig. 9). As for the C-terminal RNase H fold, this prediction was verified for a consensus sequence derived from the different Tn*3*-family transposase subgroups, reflecting the high level of sequence similarity in this region (120) (Fig. 9). Modeling is consistent with previous results that identified the IR-binding domain at the

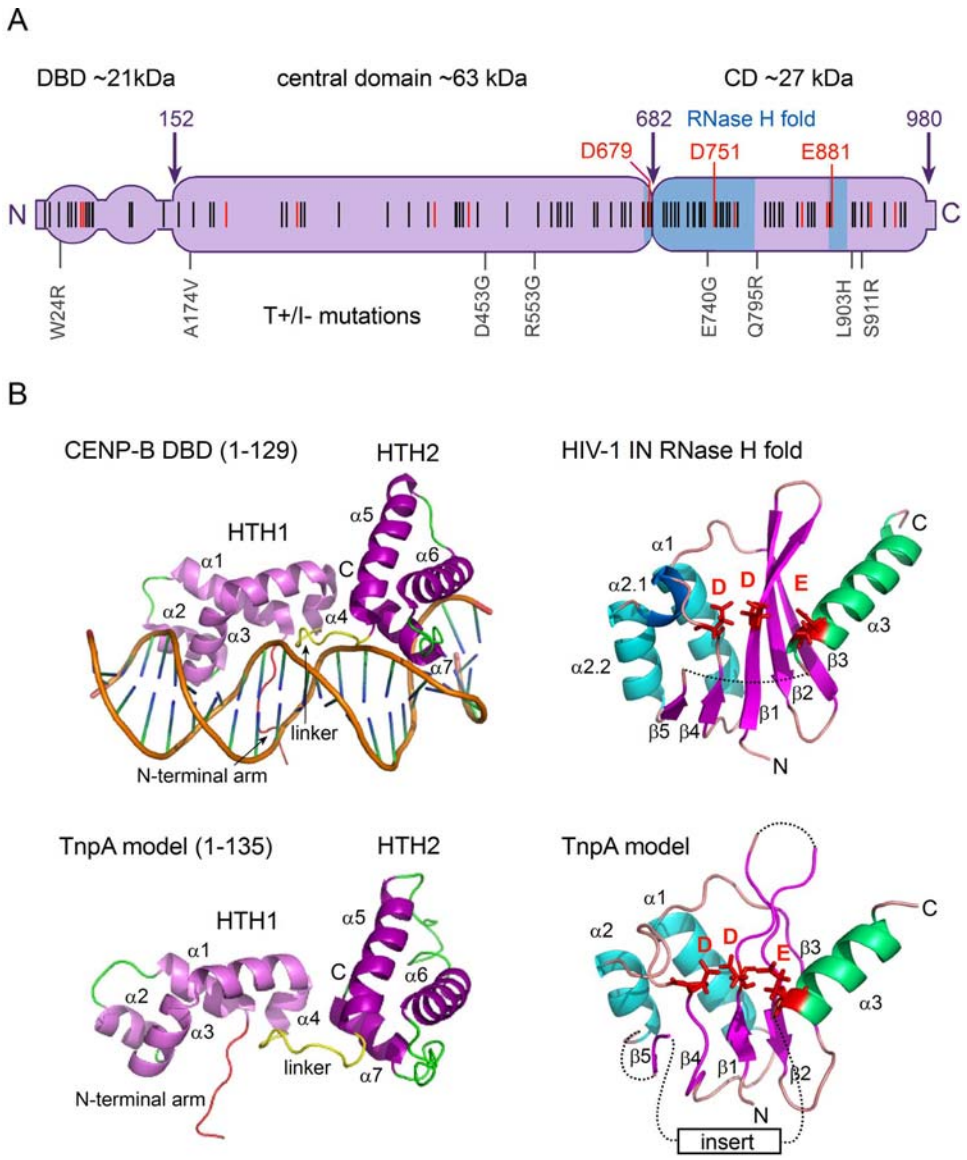


FIGURE 9 The Tn*3*-family transposase protein. (A) Structural organization of the transposase. The protein is depicted with three main domains based on limited proteolysis (vertical arrows) of the Tn*4430* transposase (987 amino acids) (120). The N-terminal DNA-binding domain (DBD) has a predicted bipartite structure analogous to that of the two-helix-turn-helix (HTH) DNA-binding domain of CENP-B. The C-terminal catalytic domain contains the predicted RNase H fold region (shaded in blue). Vertical bars correspond to highly conserved (>90%) amino acid residues in a representative subset of 21 transposases of the family, with the 15 perfectly conserved residues highlighted in red. Residues of the DD-E catalytic triad are indicated. Characterized mutations that selectively affect target immunity (T+/I– mutations) are reported below the diagram. (B) Structural models for the CENP-B-like DNA-binding domain (left panels) and the RNase H fold catalytic core (right panels). The actual structures of the CENP-B–DNA co-complex (128) and the HIV integrase RNase H fold (249) are shown on the top, and the predicted models derived for the Tn*4430* transposase are shown below. Nonstructured regions are represented by dashed lines. Positions of secondary structures and other critical structural elements are indicated and highlighted in different colors (see text for details). Nonstructured regions are represented by dashed lines. In the RNase H fold, the DD-E catalytic residues are shown in a stick configuration colored in red. The predicted location of the 90-amino acid insert in the putative RNase H fold of TnpA is indicated by a rectangle. doi:10.1128/microbiolspec.MDNA3-0060-2014.f9

N-terminus of the transposase (129, 130). Deletion of this region abolishes end binding by Tn*4430* TnpA *in vivo* and *in vitro*, while a 300-aa N-terminal fragment of the protein binds the IR end with the same affinity as the full-length transposase (E. Nicolas and B. Hallet, unpublished data).

The CENP-B DNA-binding domain consists of two helix-turn-helix motifs (HTH1 and HTH2) separated by a short, 10-aa linker (Fig. 9). Both motifs contact adjacent major DNA grooves making specific interactions with the target sequence. The N-terminal residues (residues 1 to 9) form a flexible arm that makes additional contacts with the phosphate backbone (128). These different structural components of the CENP-B binding domain appear to be conserved in the N-terminal domain of Tn*3*-family transposases based on secondary structure prediction and sequence comparison (120) (Fig. 9).

Intriguingly, the CENP-B DNA-binding domain is structurally homologous to that of Tc*1*/mariner transposon family and to the bipartite end-binding domain of bacteriophage Mu transposase, MuA (131–133). CENP-B is believed to have evolved from an ancestral transposase that has been "domesticated" during evolution to acquire a novel function (134). Confirming the presence of a CENP-B-like DNA-binding domain at the N-terminus of Tn*3*-family transposases would therefore provide an additional example illustrating the modular organization of the DD-E/D transposases, which presumably resulted from the recruitment of separate functional domains early during evolution (122, 123).

A large central domain of unknown function

In the crystal structure of the Mos*1* transposase (a Tc*1*/mariner-family member) and MuA, the N-terminal DNA-binding domain is immediately fused to the RNase H fold domain by a short connector of a few amino-acid residues (131, 133). In transposons of the Tc*1*/mariner family, this linker region contributes to the transposase dimer interface and plays a role in reciprocal communication between transposase subunits during transposition (131, 135, 136). In Tn*3*-family transposases, the equivalent region of the protein is occupied by a large central domain, corresponding to a ~63-kDa proteolytic fragment of Tn*4430* TnpA (residue 152 to 682) (Fig. 9). This domain is predicted to have an all-alpha structure. Despite several patches of well-conserved residues, it shows no sequence or structural homology with other proteins (120). However, filter binding assays performed with the Tn*3* transposase suggested that separate subregions of the central domain (residue 243 to 283 and residue 439 to 505) exhibit nonspecific DNA-binding activity (130).

The protein determinants of target immunity

A possible function of the unique central TnpA domain might be in target immunity. Most transposons and ISs do not exhibit this property and this would explain why Tn*3*-family transposases are significantly larger than other transposases. A genetic screen was developed to identify Tn*4430* TnpA mutants that could bypass immunity by promoting high levels of transposition into an immune target (i.e., a target carrying a Tn*4430* IR end). The selected T^+/I^- (i.e., transposition-plus/Immunity-minus) mutations were clustered into five discrete regions belonging to separate domains. Although two clusters of mutations were identified within the large central domain, those that showed the strongest defect in immunity mapped in the C-terminus, beyond the DD-E motif. This scattering of the T^+/I^- mutations indicates that target immunity is likely to be a complex process involving multiple activities of the transposase (120) (see also *The mechanism of target immunity: 'schizophrenic-like' behavior of the transposase*, below).

Interaction with the transposon ends

Specific transposase recognition of the transposon ends is crucial to initiate transposition on the appropriate DNA substrates and to adequately position the partners for subsequent reactions steps within the transpososome.

The conserved IR end

As for other transposable elements, the ~38-bp terminal IRs of Tn*3*-family transposons are composed of two functional domains (Fig. 10). The outermost highly conserved 5′-GGGG-3′ motif is essential for transposition, but is not required for stable TnpA binding. It contains the DNA cleavage site and is thought to be contacted by the transposase catalytic domain to carry out the chemical steps of transposition (137–142). TnpA binding to this region generally gives weak protection as determined by DNA footprinting, with some enhanced signals due to protein-induced DNA distortions around the cleavage site (140, 141, 143–148). For Tn*4430*, these signals are more pronounced upon formation of a paired-end complex, which corresponds to an activated intermediate of the transposition reaction (Nicolas *et al.*, in preparation).

The inner segment of the IR (position ~10 to 38) is the recognition domain with which the transposase makes the strongest and most specific interactions. This region also encodes the specific determinants to confer target immunity (138–140, 149, 150) (see also below). As shown in the alignment (Fig. 10), it can be further divided into two subdomains; a central AT-rich region

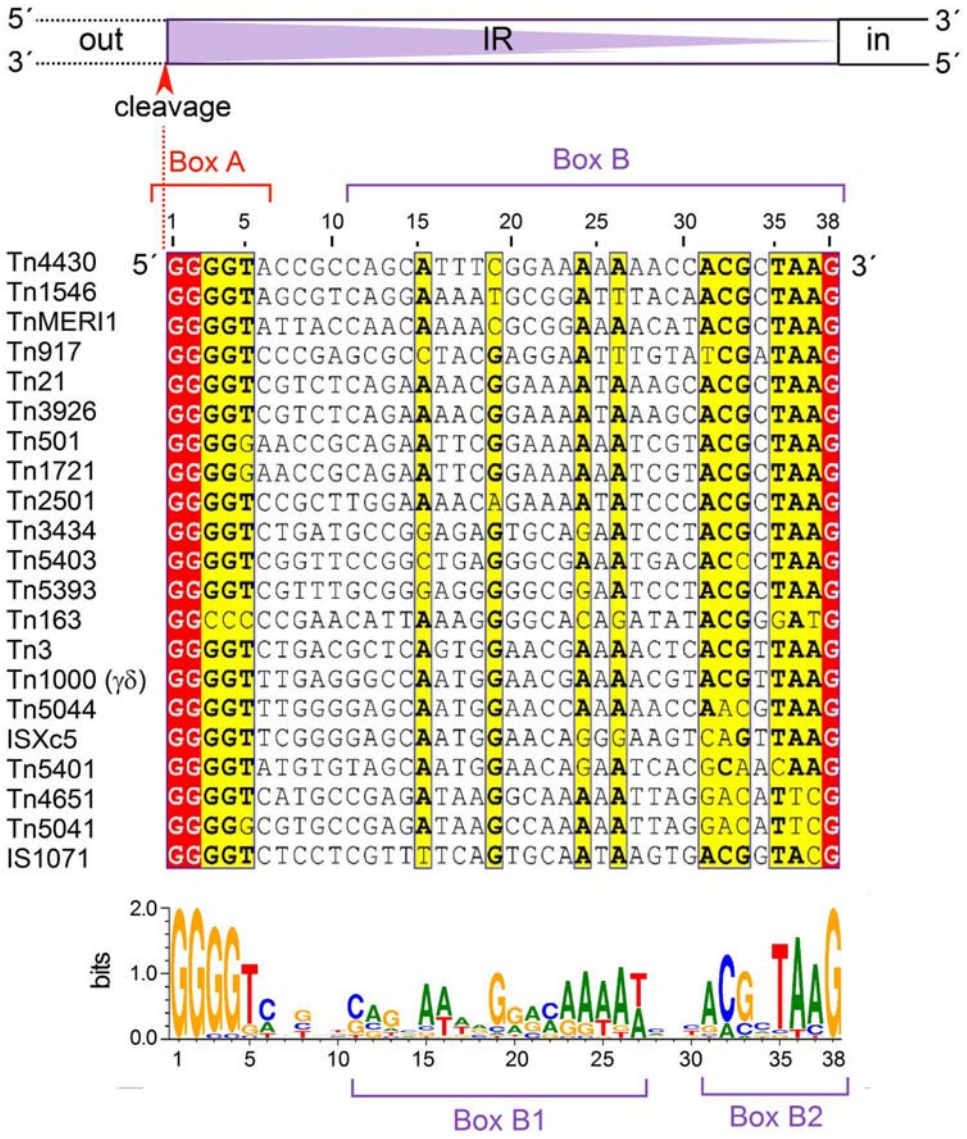


FIGURE 10 The Tn*3*-family transposons ends. The 38-bp terminal inverted repeat (IR) sequences of 21 representative transposons of the Tn*3* family are aligned, and perfectly or highly (>75%) conserved positions are boxed in red and yellow, respectively. The resulting consensus sequence is shown below the alignment, and a cartoon showing the orientation of the IR sequence (purple triangle) with respect to the inside (in) and outside (out) regions of the transposon is reported on the top. Position of the transposase 3′-end cleavage site is indicated by an arrow. Conserved regions corresponding to the external cleavage domain of the IR (Box A) and the internal transposase recognition domain (Box B) are indicated with brackets. The transposase recognition sequence is further subdivided into two conserved motifs (Box B 1 and Box B 2). doi:10.1128/microbiolspec.MDNA3-0060-2014.f10

(box B1, positions ~11 to 27) and a more conserved subdomain (box B2, position ~31 to 38) carrying the 5′-TAAG-3′ motif at the inward-facing boundary of the IR (Fig. 10). TnpA makes extended interactions with both recognition subdomains, protecting up to three adjacent major grooves (137, 141, 149). Mutations in the innermost box B2 subdomain showed the strongest defects in transposase binding and transposition activity (139, 140).

This binding pattern is consistent with the predicted CENP-B-like bipartite Tn*3*-family transposase DNA-binding domain (Fig. 9). In Tc*1*/Mariner-family

transposases and MuA, a structurally equivalent domain recognizes sequence elements of about the same length (~22 to 26 bp) as Tn*3*-family transposases (~27 bp). Their HTH motifs interact with both sides of the recognition element making specific contacts within the major groove (131–133, 151). The basic linker connecting both HTHs forms a structure termed 'AT-hook' that makes additional interactions with the AT-rich stretch that separates both HTH binding sites (131, 132). It is tempting to propose that Tn*3*-family transposases bind the transposon ends in a similar way, with one predicted HTH motif interacting with box B2 of the IR and the other, together with the linker, interacting with the longer AT-rich box B1. Additional protein segments, such as the conserved N-terminal arm of the DNA-binding domain may also contribute to the interaction, as observed in the CENP–B/DNA complex (128) (see also Fig. 9).

Extended ends: role of auxiliary proteins

A number of Tn*3*-family transposons have significantly longer terminal inverted repeats than the canonical ~38-bp IR (18, 44, 48, 65, 146, 148, 152). For some of them, this was found to correlate with the presence of additional binding sites for accessory proteins in the subterminal regions of the transposon, providing a means to control transposition at the level of transpososome assembly.

The inner segment of the 53-bp Tn*5401* terminal inverted repeats includes an extra 12-bp binding motif for the TnpI recombinase (37, 48, 148). This motif is not included in the IRS and is therefore not required for cointegrate resolution (48). By contrast, TnpI binding to this motif is essential for the formation of a stable complex between the transposase and the transposon ends (148). At the same time, as was previously reported for the resolvase of other Tn*3*-family members (7), TnpI binding to the IRS down-regulates the expression of both *tnpI* and *tnpA*, keeping transposition activity at a low level (148). This antagonistic action of TnpI may be important to ensure efficient transposition under certain circumstances. In particular, bursts of TnpI and TnpA expression would occur when the transposon enters a new host cell, before transcriptional repression by TnpI becomes effective. Functional coupling between TnpI and TnpA activities may also be regarded as a safeguard mechanism to maintain the integrity of both the transposition and cointegrate resolution modules as the transposon multiplies and spreads.

Transposase binding to the ends of γδ and Tn*4652* is influenced by the host protein IHF (integration host factor). Since the cellular abundance of nucleoid-associated proteins like IHF varies according to the growth rate (153, 154), their contribution to transposition provides a means of communicating environment signals or the physiological state of the cells to the transpososome.

For γδ, transposase and IHF binding to the transposon ends is cooperative (145). This was found to primarily impact on target immunity (155) and to rescue transposition defects resulting from specific ends mutations (139). For Tn*4652*, IHF binding is a prerequisite for transposase binding and transposition (146). IHF was also found to up-regulate Tn*4652* transposition by stimulating the transcription of the *tnpA* gene (156). TnpA expression is also under strong dependence of the stationary-phase sigma factor σ^s (RpoS) (157). Together, these different mechanisms co-operate to restrict Tn*4652* transposition under carbon starvation conditions, when the cellular concentration of IHF is maximal and σ^s is induced. Consistently, binding of Tn*4652* transposase to the transposon ends is negatively affected by Fis (factor for inversion stimulation), which unlike IHF is maximally expressed in actively growing cells (147). Fis binding to its cognate sites is proposed to out-compete IHF from the left end of Tn*4652*, thereby compromising the recruitment of the transposase. Transposition of Tn*4652* is also modulated by the *Pseudomonas putida* ColRS two-component system (158) and the small transposon-encoded protein TnpC, which appears to down-regulate the expression of the transposase at the post-transcriptional level (159).

If the requirement for specific host factors is important to correlate the rate of transposition with cell physiology, it may also restrict the host-range of transposons as was proposed for IS*1071* (152). This element is characterized by unusually long terminal IRs (110 bp) containing potential binding sites for host accessory proteins such as IHF (152).

Interaction with the target

Target site choice has strong biological implications for both the host and the transposon because it determines whether transposition will have a beneficial outcome or not. Target site specificity also reflects the molecular mechanism whereby the transposition machinery interacts with the target DNA, offering an additional level of control on transposition (160).

DNA sequence specificity and regional preference

Tn*3*-family transposons are characterized by a low level of target specificity, which likely contributed to their wide dissemination. Early studies indicated that Tn*3*-related

elements preferentially insert into specific AT-rich DNA regions (161–164). This was confirmed from large-scale analyses which revealed that the target sites of different family members conform to a similar, low-specificity consensus sequence centered on a degenerated 5-bp AT-rich core motif corresponding to the target duplications (i.e., 5′-TA[A/T]TA-3′ for Tn3, 5′-TATAA-3′ for Tn*917*, 5′-[T/A] [T/A]N[A/T] [A/T]-3′ for Tn*hopX1*, 5′-T [T/A] [T/A] [A/T] [A/T]-3′ for Tn*4652*, and 5′-[T/A]NNN[A/T]-3′, for Tn*4430*) immediately flanked by GC-rich positions (65, 165–168) (E. Nicolas and B. Hallet, unpublished data).The overall palindromic organization of this consensus likely reflects symmetrical interactions made by the transposition complex to transfer both transposon ends to the target DNA. G/C to A/T transition steps at the DNA cleavage sites may be required to distort or bend the DNA, making the target scissile phosphates more accessible for strand transfer as proposed for other transposable elements (169–171).

In all analyses, the target DNA sequence is clearly not sufficient to determine Tn*3*-family insertion specificity. Specific DNA sequences perfectly matching the consensus are poor targets, whereas other sites are targeted much more frequently than expected from their resemblance to the consensus (165, 166, 172). This suggests that integration of Tn*3*-family transposons obeys some other structural or dynamic properties of DNA.

Role of target replication

Recent work performed with Tn*4430* indicates that relatively subtle changes made in a target molecule may profoundly alter the insertion profile (173). In particular, integration of repeated copies of a pseudo-palindromic DNA sequence (i.e., three or five copies of the *E. coli lacO* operator) created strong insertion hot spots between the repeats and the unidirectional replication origin of the target plasmid (173) (E. Nicolas and B. Hallet, unpublished data). This altered regional preference did not change target sequence specificity, but was dependent on the direction of DNA replication with respect to the *lacO* repeats (E. Nicolas, C. Oger and B. Hallet, unpublished data). A plausible interpretation is that the palindromes interfere with replication forks progression in the hot spot region (e.g., through the formation of secondary structures in the DNA), thereby provoking the accumulation of preferred DNA target structures. Consistent with this, several Tn*3*-family transposons preferentially insert within or close to the replication origin of target plasmids (161, 174). On the other hand, Tn*917* transposition into the chromosome of *Enterococcus faecalis* and other Firmicutes showed a strong preference for the replication termination region (172, 175, 176). This region is subject to extensive processing, including numerous replication forks collapse and repair events (177)), which may provide the required transposition signal. Intriguingly, the Tn*4430* transposase binds *in vitro* with high affinity to branched DNA substrates that mimic a replication fork (E. Nicolas, C. Oger and B. Hallet, unpublished data).

Transposition of other transposons is also coupled to replication (178–185). Tn*7* targets the lagging strand at a replication fork (178, 179, 185) using specific interactions between the Tn*7*-encoded auxiliary protein TnsE and the β-clamp processivity factor of the replisome (186) (see also the chapter by J. E. Peters in this volume). This results in a marked preference for inserting into conjugating plasmids and is regarded as a mechanism to promote horizontal dispersal. For other transposable elements such as the IS*200*/IS*608* family and Group II introns, coupling between DNA targeting and lagging-strand DNA synthesis is inherent to the transposition mechanism that requires single-stranded DNA for integration (183, 187).

The possibility that replicative transposons like those of the Tn*3* family preferentially integrate into replication forks would provide a direct mechanism for recruiting the host replication machinery for the replicative transposition step. Indeed, experiments performed with conditionally replicating DNA molecules have shown that target replication is essential for Tn*1*/Tn*3* transposition (188). Transposition into nonreplicating target DNA was essentially undetectable, whereas blocking replication of the donor molecule had no effect on transposition activity. Tn*3*-family transposons also prefer to insert into episomes such as phages and plasmids rather than into the chromosome, presumably because the replication dynamics of these different replicons is different (189, 190).

Control of transposition by the target: target immunity

Target immunity, also called transposition immunity, is a highly selective and *cis*-acting process by which certain classes of transposable elements avoid inserting into a DNA molecule that already contains a copy of themselves. A remarkable feature of this “repulsive” process is that it can act over extended genome regions that, depending on the element, range from ~20 kb to several dozen kilobases (138, 160, 191–193). Understanding this mechanism therefore has much to tell us about how DNA is organized within the cell and how distant sites in a genome communicate with each other.

The mechanism of target immunity: "schizophrenic-like" behavior of the transposase

Target immunity was initially described for Tn*3* family members (194, 195) and subsequently for other transposable elements like Mu (196, 197) and Tn*7* (198, 199). In all cases, a single transposon end or a subdomain of the transposon terminus capable of binding the transposase is sufficient to confer target immunity (196, 197, 199, 200), but the presence of multiple transposase binding sites usually strengthens the phenomenon (120, 192, 201, 202). For Tn*3*-family transposons, the DNA determinants of target immunity coincide with the internal ~27-bp to 30-bp recognition motif (box B) of the terminal IR (120, 138–140, 149, 150) (Fig. 10).

Experiments with Tn*4430* have shown that simply attracting the transposase to a nonspecific target molecule by fusing the protein to a heterologous DNA-binding domain is insufficient to make this molecule refractory to transposition (173). This suggests that establishment of immunity requires the formation of a specific complex between the transposase and a cognate site on the target (173).

The requirement for specific interactions between the transposase and the transposon ends is only part of the self-recognition mechanism that makes each transposon of the family immunized against itself, but not against other members of the family (195, 203). This was shown in "mixing" experiments where Tn*4430* and Tn*1* were introduced into the same cells; an immune target for Tn*4430* was not immune against Tn*1* and reciprocally, a target that was immunized by Tn*1* transposase was permissive for Tn*4430* (120). The transposase of Tn*3* family transposons therefore plays a dual role in the specificity of immunity by both communicating the immunity signal to the target and by interpreting this signal.

For Mu and Tn*7*, target immunity results from the interplay between the transposase (MuA and TnsAB, respectively) and an additional element-encoded protein (MuB and TnsC) that function as a molecular "matchmaker" between the transpososome and the target (117, 118, 204–206) (see also the chapters by R. M. Harshey and J.E. Peters in this volume). Both proteins are DNA-dependent ATPases that bind to DNA in their ATP-bound form. By interacting with their partner transposase, MuB and TnsC stimulate transpososome assembly and DNA strand transfer (200, 207–210). As a result, MuB- or TnsC-bound DNA molecules are preferred substrates for Mu and Tn*7* transposition, respectively (196, 200, 208, 211–213). Interaction with the transposase also stimulates ATP hydrolysis by MuB and TnsC, which triggers their dissociation from DNA and promotes their relocation to remote sites. As this preferentially takes place on DNA molecules that carry the end-bound MuA/TnsB proteins, displacement of MuB/TnsC makes those molecules less reactive, and hence immune against further integration (196, 200, 202, 214, 215).

For Tn*3*-family transposons, the transposase is the only element-encoded protein that takes part in the immunity process. However, this does not rule out the possibility that some additional cellular function or host protein contributes by modulating transposase action in the same way for all family members despite their specific sequence and self-recognition properties. The action of transposase on the target may either be "dissuasive", by selectively restricting access to immune DNA molecules, or "attractive", by directing the transposon toward permissive targets as is proposed for the MuB and TnsC target adaptors. Depending on their global effect on transposition, the Tn*4430* TnpA T^+/I^- mutations were proposed to either impair the ability of the transposase to establish immunity, or to bypass the immunity signal (whether positive or negative) by promoting efficient transposition into nonpermissive targets (120). Supporting the latter scenario, recent biochemical data obtained with selected T^+/I^- TnpA mutants indicate that they have promiscuous activities, suggesting a possible link between target immunity and transposase activation (Nicolas *et al.*, in preparation) (see also *Catalysis of the transposition reactions*, below).

Biological roles of target immunity

Transposition immunity is traditionally presented as a strategy to favor intracellular and intercellular dispersal of transposable elements. For example, without immunity, Tn*7* would accumulate into its high-affinity chromosome site *att*Tn*7*, precluding it from spreading among bacterial populations by horizontal transfer (204, 205). Another proposed role for transposition immunity is to avoid self-destruction by autointegration (i.e., integration into itself). This is particularly crucial for bacteriophage Mu, which multiplies its genome (38.5 kb) by undergoing multiple rounds of replicative transposition in a short period of time (216). Mu target immunity is effective over ~20–25 kb on either side of the genome ends (191, 217). This would be sufficient to protect Mu against self-integration while fully exploiting the available target DNA landscape for replication.

A serious drawback of the copy-in mechanism of replicative transposition is that the transposon remains

tethered to the donor molecule through its 5′ ends after cleavage at the 3′ ends (Fig. 8). This could strongly restrict the ability of the element to reach a distant target within the cell. As a consequence, replicative transposons should exhibit a high propensity to integrate into close DNA regions belonging to the initial donor molecule. However, intramolecular transposition by the copy-in mechanism gives rise uniquely to inversions or deletions of adjacent DNA sequences, which can be detrimental for the host replicon (163, 218, 219). Therefore, the primary role of replicative transposon immunity would be to protect the host genome against such deleterious consequences of intramolecular transposition. The Tn*4430* T^+/I^- TnpA mutants provide a unique opportunity to test this possibility (120). Preliminary results indicate that these mutants mediate intramolecular transposition at a higher frequency than the wild-type transposase, correlating with their immunity defect (E. Nicolas, C. Oger and B. Hallet, unpublished data). It would be interesting to see whether this also correlates with a loss of fitness resulting from transposition-induced damages in the host replicon.

Catalysis of the transposition reactions

Our understanding of the molecular transposition mechanism of Tn*3* family transposons has long remained scant because of the technical difficulties inherent in transposase purification for biochemical characterization. An *in vitro* transposition reaction based on TnpA-enriched cell extracts was developed for Tn*3* and, as expected, TnpA was shown to introduce specific nicks at the 3′ ends of the transposon in an Mg^{2+}-dependent manner (220–222) (Fig. 8). This is consistent with TnpA being a member of the DD-E/D transposase superfamily (221, 222). However, the overall efficiency of the reaction was too low for the molecular characterization of transposition intermediates and products (220–222).

The Tn*4430* transposase was recently purified in an active and soluble form (120), allowing development of biochemical assays for the different transposition steps (from binding to the transposon ends to DNA cleavage and strand transfer; Nicolas *et al.*, in preparation). This was accomplished with the wild-type transposase and with selected immunity-deficient T^+/I^- mutants. T^+/I^- TnpA mutants are all deregulated to different extents. They spontaneously assemble an activated form of the transposition complex termed the paired-end complex in which two transposon ends are brought together by the transposase, and they exhibit higher DNA cleavage activity than the wild-type transposase. However, after cleavage, both the wild-type and mutant transposases appear to transfer the transposon ends to a DNA target with similar efficiencies. This suggests that transposition is controlled at an early stage of transpososome assembly, before initial cleavage, and that mutations that impair immunity have "unlocked" the transposase making it more prone to adopt an active configuration than wild-type TnpA (Nicolas *et al.*, in preparation).

Controlling transposition at the level of transpososome assembly is a recurrent strategy among transposable elements to ensure coordinated DNA cleavage and rejoining between appropriate partners. As the Tn*4430* T^+/I^- TnpA mutants were selected for their increased propensity to promote transposition into immune targets, it is reasonable to propose that formation of an activated paired-end complex by the wild-type transposase is normally conditioned by the target. This would provide a checkpoint allowing the transpososome to discriminate between permissive and nonpermissive integration sites. The molecular mechanism(s) that regulate(s) DNA targeting by Tn*3*-family transposons are not known, but as discussed above (see *Interaction with the target* section), an intriguing possibility is that they involve specific interactions between the transposase and cellular processes like DNA replication.

COINTEGRATE RESOLUTION BY SITE-SPECIFIC RECOMBINATION

Cointegrate resolution by Tn*3*-family S-resolvases has been the subject of several recent reviews (7, 9, 17, 31, 223, 224) (see also the chapters by W. M. Stark and P. A. Rice in this volume). Here, we will only give a brief summary of these studies, and focus on more recent work on resolvases of the Y-recombinase family.

S- and Y-resolvases: unrelated mechanisms to cut and rejoin DNA strands

S-recombinases and Y-recombinases cut and reseal DNA through the formation of a transient covalent protein–DNA intermediate involving their serine and tyrosine active site residue, respectively. For both recombinase families, DNA strand exchange takes place in the context of a tetrameric complex comprising four recombinase molecules bound onto the core recombination sites of both partners. However, the chemistry and the molecular choreography used by the S- and Y-recombinases to carry out recombination are different (17) (see also the chapters by M. Jayaram *et al.* and W. M. Stark in this volume).

S-recombinases: double-strand breaks and rotation

S-recombinases cleave all four DNA strands concurrently (Fig. 11A). This generates double-strand breaks with a two-nucleotide 3′ OH overhang on one side of the break, and a recessed end to which the recombinase is attached via a 5′-phosphoseryl bond on the opposite side. The DNA strands are then exchanged by a 180° right-handed rotation of one pair of half-sites relative to the other. Base pairing between the exchanged overhangs is necessary to properly realign the DNA duplexes and orient the DNA ends for the ligation step (Fig. 11A).

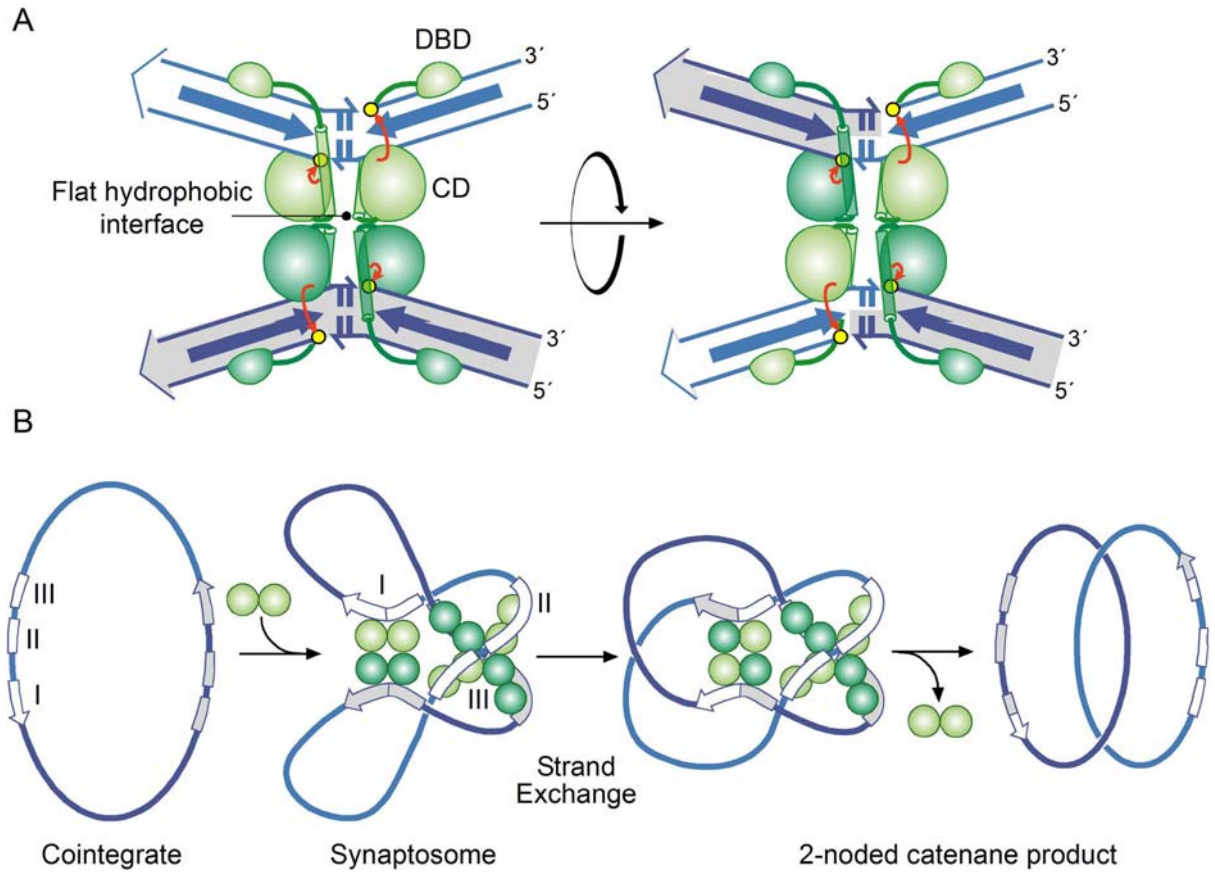


FIGURE 11 Mechanism of cointegrate resolution by resolvases of the S-recombinase family. (A) The rotational strand exchange reaction catalyzed by S-recombinases. Representation of the recombination complex is inspired from the structure of the synapse showing the activated γδ resolvase tetramer bound to paired core sites I of *res* (230, 231). The recombination sites are aligned in parallel. Blue arrows represent the 12-bp resolvase recognition motifs. The partner resolvase dimers are colored in pale and dark green with their catalytic domain (CD) lying at the inside of the synapse and their DNA-binding domain (DBD) at the outside. The four recombinase molecules have cleaved the DNA, generating double-strand breaks with phosphoseryl DNA–protein bonds at the 5′ ends (shown as yellow dots linked to red connectors) and free OH groups at the 3′ ends of the breaks (half-arrows). DNA strands are exchanged by 180° rotation of one pair of partner subunits with respect to the other around a flat hydrophobic interface within the tetramer. For the rejoining reaction, each free 3′ OH end attacks the phosphoseryl bond of the opposite DNA strand. (B) Topological selectivity in resolvase-mediated cointegrate resolution. Binding of resolvase dimers (green spheres) to sites I, II and III of the partner *res* sites results in the formation of a synaptosome in which the two *res* sites are inter-wrapped, trapping three negative crosses from the initial DNA substrate. This complex only readily forms if the starting *res* sites are in a head-to-tail configuration on a supercoiled DNA molecule. Strand exchange by right-handed 180° rotation as in (A) generates a two-node catenane product. doi:10.1128/microbiolspec.MDNA3-0060-2014.f11

This step is the reversal of the cleavage reaction, with each 3′ OH group of the cleaved ends attacking the phosphoseryl bonds in the partner (7, 9, 17, 31, 223, 224).

There has been some debate in the literature concerning the molecular mechanism of rotation (7, 225–229). The model that currently conciliates most biochemical and structural data (the "subunit rotation" model) involves a complete 180° rotation of one half of the cleaved complex relative to the opposite half (227, 230). The crystal structure of an activated form of the γδ resolvase tetramer trapped in the act of strand exchange shows that both sides of the recombinase tetramer are held together by a flat hydrophobic interface that would make such a rotational motion between recombinase subunits thermodynamically favorable without causing complete dissociation of the complex (230, 231) (Fig. 11A).

Y-recombinases: sequential strand exchange and isomerization

In contrast to S-recombinases, Y-recombinases sequentially exchange one pair of DNA strands at a time via the formation of a Holliday junction intermediate (Fig. 12A). Each strand exchange is a concerted process in which the 3′ phosphotyrosyl DNA–protein bond generated by DNA cleavage is subsequently attacked by the 5′ OH end of the partner strand. Exchange of both pairs of DNA strands implies that separate pairs of recombinase molecules in the complex are sequentially activated (Fig. 12A). Studies performed with different Y-recombinase family members have provided a detailed picture showing how this could be achieved at the molecular level (17, 232–234).

According to the model, the recombinase-bound recombination core sites are brought together in an antiparallel alignment, forming a synaptic complex with a pseudo-four-fold symmetry (Fig. 12A). The DNA is bent to expose one specific DNA strand from each duplex toward the center of the synapse for the first strand exchange. After cleavage, DNA strands are exchanged by extruding three or four nucleotides from the core central region, and by reannealing them to the partner strand to orient the cleaved 5′ OH ends for ligation. As for the S-recombinases, this "homology-testing" step ensures that appropriate DNA strands are exchanged during the reaction. The complex then isomerizes to activate the other pair of recombinase subunits for the second strand exchange (Fig. 12A).

For all Y-recombinases studied in detail, the activity of the core recombination complex is regulated by a cyclic network of allosteric interactions that places each recombinase subunit under the control of its two neighbors, so that diagonally opposed active sites of the tetramer are sequentially and reciprocally switched on and off during recombination (235–238). Isomerization of the tetramer involves relatively limited readjustments of DNA and proteins in the complex, and the mechanisms that promote these conformational changes to provide directionality to the strand exchange reaction appear to vary among different recombination systems (239).

Convergent mechanisms to control the selectivity of recombination

Although some promiscuity in resolvase-catalyzed recombination reactions may be useful from an evolutionary point of view to create new transposons (see *Mixing by recombination*, above), their primary biological function requires a preferential action on directly repeated copies of the *res* sites as they appear on a cointegrate. This is important to efficiently resolve cointegrate intermediates of the transposition process, while avoiding any other undesired DNA rearrangements.

The γδ/Tn*3* resolvase synaptsome paradigm

Efficient S-resolvase-mediated recombination of Tn*3* and γδ only takes place if two full-length *res* sites are present in the appropriate head-to-tail orientation on a supercoiled DNA molecule. Resolvase binding to the three *res* subsites (Fig. 6) promotes assembly of a multisubunit protein–DNA complex termed a synaptosome in which the two *res* sites are plectonemically interwrapped, trapping three negative supercoils from the initial DNA substrate (7, 17, 227) (Fig. 11B). The specific topology of this complex is proposed to act as a topological filter to ensure that the recombination sites are in the correct configuration (240). DNA wrapping around the synaptosome is facilitated by negative supercoiling. In contrast, formation of such a complex between inversely oriented *res* sites, or between sites on separate DNA molecules would be topologically hindered (7, 17, 227, 240).

Synaptosome assembly and activation is a dynamic process during which selective interactions between adjacent resolvase dimers result in a succession of conformational transitions that progressively drive the complex towards its final, recombination-competent configuration (17). Reciprocal interactions between resolvase dimers bound to accessory sites II and III of both *res* sites are responsible for the establishment of the initial inter-wrapped structure in which three negative nodes are trapped (241, 242). Formation of this presynaptic

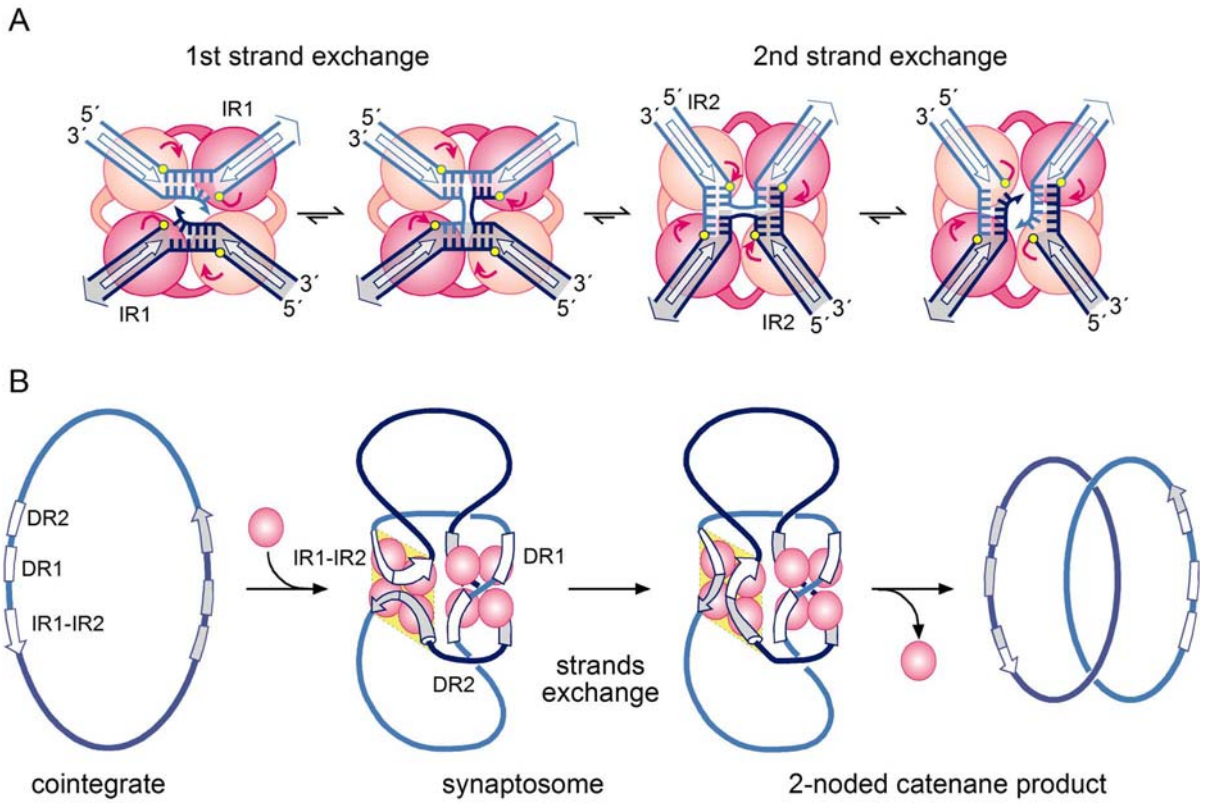


FIGURE 12 Mechanism of cointegrate resolution by the TnpI recombinase of Tn*4430*. (A) Ordered DNA strand exchange catalyzed by TnpI at the IR1–IR2 core site of the IRS. The TnpI tetramer bound to synapsed IR1–IR2 core sites is drawn according to the structure of the related Cre recombinase complexes (232). Only the C-terminal catalytic domain of the protein is shown for clarity. Each recombinase subunit is connected to its neighbors though a cyclic network of allosteric interactions that dictates its activation state during the consecutive steps of recombination. The recombination sites are brought together in an antiparallel configuration exposing one specific pair of DNA strands at the center of the synapse. In this configuration, the IR1-bound TnpI subunits (magenta) are activated to catalyze the first strand exchange and generate the Holliday junction (HJ) intermediate. The complex then isomerizes, which deactivates the IR1-bound TnpI subunits and activates the IR2-bound subunits (pink) for catalyzing the second strand exchange. For each strand exchange, the recombinase catalytic tyrosine (curved arrow) attacks the adjacent phosphate (yellow circle) to form a 3′ phosphotyrosyl protein–DNA bond, which is in turn attacked by the 5′ OH end (half-arrow) of the partner DNA strand. (B) Possible model for the topological organization of the TnpI/IRS recombination complex. TnpI binding to the DR1–DR2 accessory motifs of directly repeated IRSs generate a synaptic complex in which three DNA crosses are trapped. Proper antiparallel pairing of the IR1–IR2 core sites introduce a positive twist in the DNA so that strand exchange as in (A) generates a two-node catenane product. doi:10.1128/microbiolspec.MDNA3-0060-2014.f12

complex is the key of the topological filter that dictates whether recombination will proceed by aligning the core recombination sites I for strand exchange. Productive parallel pairing of the crossover sites I involves specific interactions between the resolvase molecules bound to site I and site III, as well as additional interactions between the site I-bound dimers of both duplexes to form a synaptic interface within the tetramer (230, 231, 243, 244).

In addition to promoting spatial juxtaposition of the crossover sites, transient interactions between the regulatory and catalytic subunits of the complex are thought to play a more direct role in the directionality of strand exchange by inducing the required conformational change

to bring resolvase from an inactive to an active configuration observed in structural studies (17, 31, 224, 230, 244). This activation step may be important to tip the recombination complex to an irreversible stage of the recombination reaction.

Because of the defined topology of the recombination complex and the rotational specificity of the strand exchange mechanism, recombination mediated by S-resolvases exclusively yields catenated molecules in which the two recombinant products are singly interlinked (Fig. 11B). The same product topology has been reported for other resolution systems using S-recombinases suggesting a common topological structure of the synaptic complex even if the organization of the recombination sites and the molecular architecture of the synaptosome are different (19, 25, 27, 30, 31) (see also the chapter by P. A. Rice in this volume).

Variation on a theme: TnpI/IRS recombination complex of Tn*4430*

A mechanism of toplogical selectivity analogous to that described for the serine recombinases controls cointegrate resolution mediated by the Tn*4430* Y-recombinase, TnpI (47). As for S-resolvases, TnpI mediates recombination without additional host factors, acting both as a catalytic and as a regulatory component of the recombination complex. However, as opposed to most regulated site-specific recombinases, TnpI does not absolutely require the DR1 and DR2 accessory binding motifs of the resolution site IRS to be active (47) (see also Fig. 7). In the absence of DR1 and DR2, TnpI-mediated recombination at the IR1–IR2 core site is nonselective (or "unconstrained") giving rise to all possible DNA rearrangements *in vivo* (i.e., deletions, inversions, or intermolecular fusions) and to topologically complex products *in vitro* (47). In contrast, the DR1 and DR2 accessory motifs stimulate intramolecular recombination between directly repeated IRSs and generate exclusively two-node catenane products *in vitro* (47) (Fig. 12B).

More recent topological analyses indicate that TnpI binding to DR1 and DR2 results in the formation of a complex in which the accessory sequences are interwrapped approximately three times. As for the S-resolvases, formation of this complex acts as a checkpoint (i.e., topological filter) to ensure that the recombination sites are in a proper head-to-tail configuration. Changing the arrangement between the core site and the accessory motifs inhibits recombination by compromising productive core site pairing or forcing alignment in an incorrect configuration, while mutational inactivation of DR1 and DR2 increases the level of topologically unconstrained recombination arising from random collision of the recombination sites (47, 245) (C. Galloy, D. Dandoy and B. Hallet, unpublished data).

The formation of two-node catenanes as the unique products of recombination also implies that the topologically constrained synapse between the TnpI-bound DR1–DR2 sequences imposes a specific alignment of the IR1–IR2 core sites to carry out DNA strand exchange. This alignment is such that antiparallel pairing of the core sites introduces a positive twist in the synaptic complex, thereby compensating for one negative node trapped by the accessory sequences (Fig. 12B). Specific positioning of the core sites within the recombination complex correlates with a defined order of activation of the catalytic TnpI subunits within the tetramer (245). The most distant core subunits with respect to the accessory sites (i.e., the IR1-bound subunits in the wild-type IRS) initiate recombination by catalyzing the first strand exchange, while the proximal subunits (i.e., the IR2-bound subunits) resolve the Holliday junction intermediate by exchanging the second pair of strands (245) (Fig. 12B).

According to current models, directionality of strand exchange depends on the bending direction of the core sites as they are assembled in the synapse. There are two possible recombinase tetramer configurations in which diagonally opposed recombinase subunits are activated for catalysis (Fig. 12B). If one configuration is used to initiate recombination, then the opposite configuration of the complex will terminate the reaction following isomerization (245). In the TnpI/IRS system, the choice of starting recombination with one configuration of the synapse instead of the other is primarily dictated by the DR1 and DR2 accessory motifs. In their absence, unconstrained recombination catalyzed by TnpI at the IR1–IR2 core site takes place with both possible synapse configurations and no preferred order of strand exchange (245).

Based on structural data reported for other tyrosine recombinases, positioning of the TnpI core tetramer in the synaptic complex would orient the recombinase C-terminal domains toward the regulatory region of the complex, while the N-terminal domains of the TnpI core subunits would point away from the accessory sequences (Fig. 12B). Selecting for this specific arrangement of the core complex may occur indirectly by imparting a specific path to the core sites, or more directly by requiring specific interactions between the core and accessory TnpI subunits of the complex. Imposing a specific pairing of the core recombinase complex to start recombination is

important to avoid the formation of unproductive (i.e., parallel) synapses (245).

In addition to providing an architectural scaffold for assembly of the topologically and functionally selective synapse, the DR1–DR2 accessory sequences of Tn*4430* IRS also affect recombination directionality by acting at later steps in strand exchange (245). Their correct orientation in the recombination substrate stabilizes DNA cleavage and rejoining intermediates that could not be observed in reactions using the minimal IR1–IR2 core site alone (245). Cleavage of the first pair of DNA strands is proposed to release the free energy stored in the topologically constrained synapse to bring about conformational changes required to generate the Holliday junction intermediate and to promote its subsequent isomerization (245). This allosteric activity of the accessory components of the complex ensures efficient substrate conversion to products by preventing reversal of the reaction.

A topologically defined complex with a different molecular architecture promotes selectivity during resolution of plasmid multimers by the tyrosine recombinase XerCD from *E. coli* (39, 246). In this case, assembly of the topologically constrained synapse requires dedicated cellular proteins instead of extra recombinase molecules and the recombination product is a four-node catenane instead of a two-node catenane. It is presently unclear whether similar mechanisms of selectivity function in the TnpS–TnpT/*rst* (OrfQ-OrfI/*att5041*) cointegrate resolution system encoded by the Tn*4651*/Tn*5041* subgroup of Tn*3*-family transposons (41, 43, 44) (Fig. 7). Recombination mediated by the tyrosine recombinase TnpS at the *rst* resolution site of Tn*4651* is a relatively slow process that requires the accessory protein TnpT (44). However, it is not known whether TnpT binding to *rst* results in the formation of a topologically constrained synaptic complex as for other resolution systems. Likewise, further biochemical studies are required to decipher the molecular mechanisms that control recombination mediated by the PmrA-like tyrosine recombinase encoded by Tn*4556* (7) and by the TnpI/*attI* recombination system of Tn*4655* (247).

CONCLUSIONS AND PERSPECTIVES

Since their initial identification as the first antibiotic resistance transposons, isolation of new members of the Tn*3* family has continued to demonstrate their constant implication in the tit-for-tat race between bacterial pathogens and humans since the onset of antimicrobial therapies. However, both the collection and dissemination of antibiotic resistances among pathogens, and the emergence of bacterial isolates with new catabolic capabilities in polluted environments are clearly the result of recent adaptation to selective pressures imposed by human activities. In nature, Tn*3*-family transposons are likely associated with a much broader range of accessory functions, and their real impact on bacterial adaptability remains underestimated. Of particular interest is the recent finding that these transposons can promote the mobility of potentially highly versatile elements such as MITEs and MICs. Because of their minimal structure, these elements inevitably eluded classical genome annotation and thorough genomic surveys are therefore required to assess their contribution to bacterial diversification and phenotypic adaptation. Further comparative studies of both autonomous and nonautonomous elements are also necessary to decipher the mechanisms of functional module acquisition and reshuffling within and between separate subgroups of the family.

The biological relevance of Tn*3*-family transposons sharply contrasts with our current understanding of the molecular mechanisms that mediate and regulate their mobility. Most of what we known about the copy-in replicative transposition comes from studies on bacteriophage Mu, and it is presently not known whether the Mu paradigm applies to more "conventional" replicative transposons such as those of the Tn*3* family. Tn*3*-family TnpA proteins are only distantly related to other DDE-D transposases and contain a large and structurally unique central domain that is not found in other proteins. In contrast to Mu and Tn*7*, TnpA is the only transposon-specific protein involved in both transposition and target immunity, and the self-recognition mechanism whereby this single protein imposes and responds to the immunity signal remains an enigma. Finally, integration of Tn*3*-family transposons appears to depend on target DNA replication, suggesting a possible mechanism to synchronize transposition with DNA synthesis. These different aspects of the transposition mechanism raise a number of new working hypotheses that can now be addressed at the cellular and molecular levels by using newly developed genetic and biochemical tools. The prospect of these studies will be to provide an integrated view connecting transpososome assembly and activation with target immunity and DNA replication.

ACKNOWLEDGMENTS

We are grateful to P. Siguier for her expertise in protein sequence comparison and for her help in building the phylogenetic trees. We also thank A. Varani and M. Chandler for sharing data before its publication. Work in the laboratory of B.H. is supported by grants from the Fonds National de la Recherche Scientifique

(FNRS) and the Fonds Spéciaux de La Recherche (FSR) at UCL. M.L., D.D., C.G. and C.O held a FRIA fellowship and E.N. was research assistant at the FNRS. B.H. is honorary senior research associate at the FNRS.

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