Autonomous and non-autonomous Tn3-family transposons and their role in the evolution of mobile genetic elements

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The Tn3 family of transposons includes diverse elements that encode homologous transposases and contain conserved terminal inverted repeat sequences (IRs). The recent identification of non-autonomous elements, named TIMEs (Tn3-derived Inverted-repeat Miniature Elements), has shed new light on the diversity and evolution of this transposon family. A common feature of TIMEs and other members of this family is their ability to mobilize genomic DNA for transposition as part of composite transposons. These elements significantly influence the structure and properties of plasmids and other mobile genetic elements (MGEs). They may contain and move by transposition (i) plasmid replication systems, (ii) toxin-antitoxin systems and (iii) site-specific recombination modules that can resolve plasmid multimers. Some Tn3 family elements may also transfer large segments of chromosomal DNA into plasmids, which increases the pool of mobile DNA that can take part in horizontal gene transfer.

their genetic organization: (i) class 1 transposons, gathering insertion sequences¹ and related elements generated by ISs (i.e. composite transposons and ISCR/TMo elements^{2,3,4}) and (ii) class 2 transposons with more complex transposition machinery, gathering mainly the members of the Tn3⁵ and Tn7^{6,7} transposon families. A separate group of TEs consists of small (generally less than 300 bp) non-autonomous elements, whose transposition is fully dependent on trans-acting compatible transposases encoded by related functional TEs co-residing in a cell. These elements are mainly represented by MITEs (Miniature Inverted-repeat Transposable Elements), which (as judged from bioinformatic sequence analyses) are abundant in many bacterial genomes, although in the vast majority of cases their transposition activity has not been experimentally confirmed.⁸

Diversity of Tn3-Family Transposons

Our research has largely focused on the identification and functional characterization of Tn3 family elements, which are the most abundant group of bacterial transposons. These elements are characterized by the presence of genes encoding large highly related transposases. The Tn3 family comprises (i) insertion sequences and (ii) diverse non-composite transposons (classified to Tn3, Tn501, Tn4430 and Tn5652 subfamilies), containing a site-specific recombination module (composed of a resolvase gene and a *res* sequence) responsible for the resolution of cointegrates (at the *res* sites), which are intermediate forms in replicative

Transposable elements (TEs) are components of nearly all bacterial genomes. Their activity leads to the generation of various mutations and DNA rearrangements (such as insertions, deletions, duplications, inversions and translocations) and replicon fusions. These highly recombinogenic elements can also mobilize chromosomal genes for transposition, which enriches the pool of mobile DNA involved in horizontal gene transfer. Overall, the presence and activity of these elements leads to the significant acceleration of bacterial evolution. TEs have been divided into 2 main groups according to

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transposition.⁵ We have analyzed functional TEs of the Tn3-family in a pool of environmental strains of the genera *Paracoccus* (*Alphaproteobacteria*)⁹ and *Pseudomonas* (*Gammaproteobacteria*).¹⁰ These elements were identified using trap plasmids which enable positive selection of transposition events and therefore the direct “capture” of even phenotypically silent elements.¹¹ This approach permitted the characterization of several typical non-composite transposons: (i-ii) Tn3434 and Tn6122 – cryptic elements^{9,12}, (iii) Tn5393 – conferring streptomycin resistance^{9,12,13} (iv) Tn4662a – containing a predicted toxin-antitoxin module, and (v) Tn5563a – carrying part of the *mer* mercury resistance operon.¹⁰ Interestingly, we also identified several TEs of unusual structure, which significantly broaden our knowledge on the diversity and evolution of Tn3 family transposons. These atypical elements are (i) a composite transposon Tn*Ppa1* of *Paracoccus pantotrophus* DSM 11072 (*Alphaproteobacteria*)¹⁴ and (ii) a group of non-composite transposons of diverse structure isolated from *Pseudomonas* spp.¹⁰

Tn*Ppa1* (44,286 bp) is composed of 2 divergently oriented copies of cryptic transposon Tn3434 (Tn3 family), which border a large DNA region of chromosomal origin, containing genes encoding enzymes involved in the central intermediary metabolism, plus transporters, transcriptional regulators and conserved proteins of unknown function^{9,14}. This element may be considered a composite transposon whose transposition is mediated by 2 copies of the non-composite Tn3434. Besides its structure, an intriguing feature of this element was its unusually high frequency of transposition into incoming plasmids. Interestingly, this transposition phenomenon was dependent on the nature of the incoming replicon.⁹

The second group of atypical “captured” TEs are non-autonomous Tn3-family elements, named TIMEs (Tn3-derived Inverted-repeat Mobile Elements), identified in several *Pseudomonas* spp. strains. Although these small elements (262 bp) lack a transposase gene, they possess characteristics typical of the Tn3 transposon family¹⁰: (i) they contain signature Tn3 family terminal inverted

repeat sequences (IRs) of 38 bp in length, with a conserved outer 5'-GGGG-3' sequence flanking the entire element (highly homologous to IRs of Tn3 family transposon Tn5563), (ii) they transpose into AT-rich target sequences, (iii) their insertion is associated with the generation of 5- or 6-bp-long direct repeats (DRs), and (iv) they seem to preferentially transpose into plasmid molecules, since the majority of related non-autonomous elements found in sequence databases are located within such replicons.

A bioinformatic database survey revealed other non-autonomous elements, which originate from different progenitor Tn3-family transposons. Since they constitute a distinct group of elements, we suggest the establishment of a TIME family, gathering all elements that can be mobilized for transposition by Tn3-family transposons.¹⁰

We also demonstrated that TIMEs have the ability to generate larger more complex non-autonomous elements (TIME-COMP) resembling IS- or Tn3434-driven composite transposons in structure. Moreover, use of a trap plasmid identified a TIME derivative (TIME-RES) containing a site-specific recombination system homologous to that of the Tn3-family transposon Tn5563.¹⁵

Our findings permit a reassessment of the direction and dynamics of the evolution of Tn3-family TEs. It is generally accepted that these elements originated from a progenitor insertion sequence. The archetype of these elements is IS1071 of *Alcaligenes* sp.¹⁶ and around 30 related ISs have been identified in different taxonomic groups of bacteria (ISfinder database; <https://www-is.biotoul.fr>).

An important step in the evolution of Tn3-family elements was the incorporation of a site-specific recombination system (RES) into the ISs, which significantly affected the dynamics of the transposition process by ensuring the effective resolution of co-integrates. Examples of such elements are Tn6122 (3792 bp) of *Paracoccus halophilus* JCM 14014 and the aforementioned Tn3434 (3659 bp), responsible for mobility of Tn*Ppa1*.⁹ Both elements encode related transposases, but their resolvases are more divergent, suggesting that different RES

modules might have been independently acquired in the course of evolution. This was confirmed by the identification of Tn3-family transposons (e.g. Tn4430¹⁷), whose RES modules encode proteins that are highly related to phage integrases. Identification of the aforementioned TIME-RES element provides evidence that site-specific recombination systems may also be acquired by means of transposition. The acquisition of additional genes, often conferring new properties to the host strain (e.g., heavy metal and antibiotic resistance, the ability to degrade various organic compounds), has led to the creation of a variety of non-composite transposons. Genomic rearrangements and the acquisition of additional genetic information, including numerous house-keeping genes, often embedded within other types of TEs, has led to the formation of very large TEs, which may be considered transposable genomic islands (TGEI).^{14,18}

TIME elements represent the products of evolution of Tn3 family elements in the opposite direction, since they were formed by reduction of the ISs or non-composite transposons. Their mobility is fully dependent on the presence of related autonomous elements in the cell.

An interesting feature of TIMEs, as well as Tn3-family ISs and non-composite transposons, is their ability to mobilize genomic DNAs to generate diverse composite elements. This property, which is typical of insertion sequences, points to the origin of these elements.

In conclusion, our findings show that the Tn3-related transposons are much more diverse than previously thought and this family contains examples of all the structural types of bacterial TEs described so far. Similar diversity has been reported for certain prokaryotic IS elements and eukaryotic transposons.^{3,4,19-24}

The Influence of Tn3-Family Elements on the Structure and Properties of Plasmids and Other Mobile Genetic Elements

TEs of the Tn3 family preferentially transpose into bacterial plasmids, which influences both the structure and

properties of these replicons. Plasmids differ highly in respect of their genetic content. They are composed of different combinations of genetic modules responsible for plasmid replication, stabilization (partitioning, toxin-antitoxin and multimer resolution systems) and conjugal transfer. They also contain diverse accessory genetic information, which may potentially influence the phenotype of the host. Their “patchwork” structures result from recombinational shuffling of genetic information between different replicons co-residing in a cell. Tn3-family transposons may play an important role in this process, since they have the potential to mobilize DNA segments of plasmid origin as a part of composite elements.

We showed that non-autonomous TIMEs are able to transfer the replication system (REP) of a natural plasmid pZM1P1 of *Pseudomonas* sp. in this way. Transposition of the resulting composite element (TIME-COMP2) into other plasmids generates composite replicons, with a potentially broadened host range. It is probable that TIME-COMP2 (as well as analogous composite elements) may also transpose into integrative and conjugative elements (ICEs) and integrative and mobilizable elements (IMEs), which primarily reside in a host chromosome but can excise and form circular molecules that are intermediates for conjugal transfer. In contrast to plasmids, ICEs and IMEs do not contain their own replication systems. Therefore transposition of composite TIMEs into these elements may lead to the creation of novel conjugative or mobilizable plasmids.

Analogous composite elements may also promote the transfer of other plasmid-encoded genes and modules. The confirmed presence of a functional promoter within a TIME may enable efficient expression of the transferred genes in other hosts.

Non-composite Tn3-family transposons may also play an important role in shaping the properties of bacterial plasmids. It has been demonstrated experimentally that the RES modules of these elements are functional analogs of plasmid-encoded multimer resolution systems (MRS). Therefore, an anchored transposon may ensure or increase the stability of

the host replicon by providing a site-specific recombination module, enabling resolution of plasmid oligomers.²⁵ An intriguing example is the non-autonomous TIME-RES element, which may serve as a portable RES system. It is highly probable that the transposition of such elements into different ISs may produce novel, dynamic TEs with co-integrate resolution properties typical of Tn3-family elements.

Interestingly, in addition to RES, one of the *Pseudomonas* spp. transposons that we identified (Tn4662a) also carries a TA module ensuring plasmid “addiction” by killing plasmid-less cells. This module is highly homologous to the *tad-ata* system of plasmid pAM12 of *Paracoccus aminophilus*.²⁶ Thus, the Tn4662a is probably able to ensure much more efficient stabilization of the host plasmid, also at the post-segregational level.

The TA-containing transposons may also stabilize elements such as prophages and other integrative elements, including ICEs and IMEs. ICEs and IMEs can temporarily exist in bacterial cells in an autonomous form, excised from the donor site. Since their circular, plasmid-like forms are present as single copies and they are unable to replicate, cell division results in the loss of these elements. Therefore, the acquisition of a TA module, which eliminates cells lacking autonomous forms of elements from the population, seems to be evolutionarily advantageous for these elements. Such a stabilizing effect has been shown for the SXT element of *Vibrio cholerae*.^{26,27}

TEs of the Tn3 family often contain an additional genetic load. Many are large elements that may constitute a significant portion of a plasmid genome. Their presence and transposition may influence the properties of both the host replicon and bacterial strain. Such transposons can confer a variety of phenotypes, enabling adaptation of host strains to various ecological niches.

Using trap vectors we have observed highly dynamic transposition of several TEs, including Tn5393, conferring streptomycin resistance,^{9,12,13} and TnPpa1, which delivers a huge genetic load of chromosomal origin into the plasmid.^{9,14} Such intracellular transfer of chromosomal

housekeeping genes may lead to the generation of chromids – essential replicons sharing features of both plasmids and chromosomes.^{28,29} Tn3-family transposons may target all types of mobile genetic element. Besides plasmids, they are found in (i) prophages (e.g. P7 contains Tn3 conferring ampicillin resistance), (ii) integrative elements (e.g., IME named genomic island SGI1 contains remnants of 3 types of Tn3-like TEs), and (iii) within other TEs, which results in the generation of complex elements with mosaic structures (e.g. Tn21).^{30,31}

To conclude, TEs of the Tn3 family are highly diverse elements with a huge evolutionary potential. They shape the structures and influence the properties of many mobile genetic elements. The identification and characterization of diverse non-autonomous transposons (TIME, TIME-COMP, TIME-RES) has expanded the Tn3 family of elements and increased our understanding of the evolution of this group of TEs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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