Traveler, a New DD35E Family of Tc1/Mariner Transposons, Invaded Vertebrates Very Recently

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Abstract

The discovery of new members of the Tc1/mariner superfamily of transposons is expected based on the increasing availability of genome sequencing data. Here, we identified a new DD35E family termed Traveler (TR). Phylogenetic analyses of its DDE domain and full-length transposase showed that, although TR formed a monophyletic clade, it exhibited the highest sequence identity and closest phylogenetic relationship with DD34E/Tc1. This family displayed a very restricted taxonomic distribution in the animal kingdom and was only detected in ray-finned fish, anura, and squamata, including 91 vertebrate species. The structural organization of TRs was highly conserved across different classes of animals. Most intact TR transposons had a length of ~1.5 kb (range 1,072–2,191 bp) and harbored a single open reading frame encoding a transposase of ~340 aa (range 304–350 aa) flanked by two short-terminal inverted repeats (13–68 bp). Several conserved motifs, including two helix-turn-helix motifs, a GRPR motif, a nuclear localization sequence, was only detected in ray-finned fish, anura, and squamata, including 91 vertebrate species. The structural organization of TRs was highly conserved across different classes of animals. Most intact TR transposons had a length of ~1.5 kb (range 1,072–2,191 bp) and harbored a single open reading frame encoding a transposase of ~340 aa (range 304–350 aa) flanked by two short-terminal inverted repeats (13–68 bp). Several conserved motifs, including two helix-turn-helix motifs, a GRPR motif, a nuclear localization sequence, and a DDE domain, were also identified in TR transposases. This study also demonstrated the presence of horizontal transfer events of TRs in vertebrates, whereas the average sequence identities and the evolutionary dynamics of TR elements across species and clusters strongly indicated that the TR family invaded the vertebrate lineage very recently and that some of these elements may be currently active, combining the intact TR copies in multiple lineages of vertebrates. These data will contribute to the understanding of the evolutionary history of Tc1/mariner transposons and that of their hosts.

Key words: Tc1/mariner transposons, Traveler, DD35E, horizontal transfer, evolution.

Introduction

Transposable elements (TEs) are mobile DNA fragments in host genomes that are able to change their genetic environment and act as major factors that contribute to the evolution of genomes; they also play an important role in genomic structure and genetic innovation (Feschotte and Pritham 2007; Huang et al. 2012). TEs are distributed extensively in both eukaryotes and prokaryotes; however, they are far more abundant in eukaryotic genomes. Furthermore, in both prokaryotes and eukaryotes, there seems to be a direct positive correlation between genome size and TE abundance (Kidwell 2002; Touchon and Rocha 2007). TEs are major determinants of genome size (Hawkins et al. 2006; Gao et al. 2016). Forty-five per cent of the human genome consists of TEs (Lander et al. 2001) versus nearly 85% in the maize genome (Schnable et al. 2009). TEs are classified into two types (classes I and II) according to their mechanism of transposition. Class I elements (retrotransposons) are transposed via the reverse transcription of an RNA intermediate. Class II elements (DNA transposons) can be further divided into three major subclasses: the classical “cut-and-paste” DNA transposons, “rolling circle” DNA transposons, and “self-synthesizing” DNA transposons (Feschotte and Pritham 2007). Despite the differences in transposition mechanisms, some integrases of RNA TEs and transposases of DNA TEs are thought to have a common origin (Capy et al. 1997).

The Tc1/mariner superfamily is a “cut-and-paste” group of class II TEs that was first discovered in Drosophila mauritiana (mariner) (Jacobson et al. 1986) and Caenorhabditis elegans (transposon C. elegans number 1, Tc1) (Emmons et al. 1983) and is distributed extensively in eukaryotes (Haymer and Marsh 1985; Jacobson et al. 1986). The Tc1/mariner transposons generally have a size of 1,300–2,400 bp and encode a 340 amino acid (aa) transposase that is flanked by two terminal inverted repeats (TIRs) and dinucleotide target site duplications (TSDs) of TA (Lohe et al. 1996). Diverse families of this superfamily, such as DD34E/Tc1, DD34D/mariner, DD36E/D, DD37D/maT, DD37E/TRT, DD39D, DD41D, DD41D/pogo, and...
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DD×E, have been defined based on the phylogeny of the DDE conserved catalytic motif (Shao and Tu 2001; Bouuaert et al. 2015; Sang et al. 2019). DD34E/Tc1 (Vos et al. 1993; Radice et al. 1994; Lam et al. 1996; Iivics et al. 1997; Sizelle et al. 2005), DD×Dipogo (Tudor et al. 1992), and DD34D/mliner (Robertson 1993; Plasterk et al. 1999; Arkhipova and Meselson 2005; Nguyen et al. 2014) have been known for a long time and have been studied extensively, whereas DD37D/ma7 (Robertson and Asplund 1996; Gilchrist et al. 2014), DD39D (Jarvik and Lark 1998; Tarchini et al. 2000), and DD41D (Gomulski et al. 2001) were identified recently, with few reports being available; however, their evolution profiles, including taxonomic distribution, intrafamily diversity, and evolutionary dynamics in genomes are poorly understood. In contrast, DD36E/C (Sang et al. 2019) and DD37E/TRT (Zhang et al. 2016) are newly discovered families with well-defined evolution profiles. DD37E/TRT was confirmed as a new subfamily within the Tc1/mariner superfamily and is present in bony fishes, the clawed frog, snakes, protozoans, and fungi; this widespread distribution of DD37E/TRT among fishes, frogs, and snakes is the result of multiple independent horizontal transfer (HT) events. DD36E/Incomer represents a unique DD36E motif that seems to have originated from DD34E and is mainly distributed across vertebrates (including jawless fish, ray-finned fish, frogs, and bats), with a restricted distribution in invertebrates (four species in Insecta and nine in Arachnida). HT events of DD36E/C were also detected in vertebrates (Sang et al. 2019). In addition, new monophyletic clades of DD34E (termed Gambol) (Coy and Tu 2005) and DD37E (termed Tnp) (Puzakov et al. 2018) were identified that are distinct from the previously discovered DD34E/Tc1 and DD37E/TRT families and form separate branches.

The Traveler (TR) elements were first discovered in the Salmo salar genome via a TBLASTN search using the sleeping beauty (SB) transposase (Iivics et al. 1997), which is a well-known DNA transposon of the Tc1/mariner superfamily. The intact TR element in S. salar has the typical structural organization of Tc1/mariner transposons, with TIRs flanking the segments of the transposon (~1.5 kb) and transposase (338 aa); however, it comprises a unique DD35E motif (fig. 1) that differs from the typical DDE motif (DD34E) of the Tc1 family (Lam et al. 1996), indicating that TR is a potential new family of Tc1/mariner transposons. To illustrate the evolution profiles of TR in genomes, we investigated the taxonomic distribution, structural organization, phylogenetic nature, and amplification dynamics of TRs. Our data revealed that TR is a new family that evolved recently from DD34E/Tc1 and exhibits a restricted taxonomic distribution in vertebrates and recent invasion events in most detected lineages. Our study also identified multiple HTs of TRs in vertebrates. Overall, we discovered a unique DD35E transposon family, which expanded the diversity of the Tc1/mariner superfamily, thus promoting the understanding of the evolution of DNA transposons and their impact on animal genomes.

Materials and Methods

Retrieval of TR Elements
To assess the distribution of TR elements in genomes, the TR transposase sequence of S. salar was used to search the whole-genome shotgun contig database at the NCBI using TBLASTN with a value of $1e^{-100}$. This transposon was manually determined to exist in a species when the catalytic domain (DD35E) of TR was detected. Significant hits were extracted with 1,000-bp flanking sequences, which were aligned to determine their boundaries. Subsequently, the representative sequence or consensus sequence of TR was searched against its host genome, to estimate copy number. All hits obtained that were >1,000 bp in size and had 80% identity were used to calculate the copy number. The consensus sequence of TR was reconstructed. In addition, transposons with a low copy number in the genome, which may be false-positive hits resulting from sequence contamination in the assembled genome or WGS, were verified further by mapping the flanking sequences of the transposon insertion to the host genome or to the genomes of closely related species; the unmapped transposons were designated as sequence contamination and were excluded from the analysis.

Sequence Analysis and Phylogenetic Inference
Protein secondary structure predictions were performed using the PSIPRED program (http://bioinf.cs.ucl.ac.uk/psipred/) (McGuffin et al. 2000). Putative nuclear localization signal (NLS) motifs were predicted using PSORT (https://www.psort.org) and predicted using the PSIPRED program (http://bioinf.cs.ucl.ac.uk/psipred/). Multiple alignments were performed using the multiple alignment program ClustalW embedded in the BioEdit tool (Yang et al. 2003) and were manually edited and annotated using GeneDoc (Nicholas et al. 1997). The protein domains were identified using the profile hidden Markov Models with the online hmmscan web server (https://www.ebi.ac.uk/Tools/hmmer/search/hmmscan). TIRs were manually determined using the ClustalW program in the BioEdit tool. The consensus sequence was constructed using DAMBE (Xia 2018). A consensus sequence or representative sequence of each identified transposon was selected for further analysis in this study. The species divergence times were estimated using the online TimeTree program (http://www.timetree.org) (Hedges et al. 2015). Sequence identities between the TR family and other families were measured via pairwise comparisons of full-length (FL) transposases using the BioEdit tool. The conserved DDE domains of the identified TR transposases and FL transposases were aligned to the representative TE families from the Tc1/mariner superfamily separately using MAFFT v. 7.310 (Yamada et al. 2016). The phylogenetic trees were inferred based on the conserved DDE domain (~150 aa) (supplementary data set S1, Supplementary Material online) and the FL Tc1/mariner transposases (supplementary data set S2, Supplementary Material online).
online) using the maximum likelihood method with the IQ-TREE program (Nguyen et al. 2015). The best-suited aa substitution model for these data was the VT+I+G4 model, according to BIC, which was selected by ModelFinder (Kalyaanamoorthy et al. 2017). The reliability of the maximum likelihood trees was estimated using the ultrafast bootstrap approach with 1,000 replicates.

### Pairwise Distances between the TR and RAG1 Sequences

Pairwise distances between the different animal species included in this study were calculated for the TR and RAG1 coding sequences, to test the HT hypothesis. Their accession numbers are listed in supplementary table S1, Supplementary Material online. Species for which we were unable to find the complete CDS region of the RAG1 gene in the NCBI database were excluded from the analysis. Multiple alignments of RAG1 (supplementary data set S3, Supplementary Material online) and TR (supplementary data set S4, Supplementary Material online) were generated using the MUSCLE program embedded in MEGA (v. 7.2.06) and were used to calculate the pairwise distances using MEGA (v. 7.2.06) (pairwise deletion, maximum composite likelihood) (Kumar et al. 2016).

### Evolutionary Dynamics Analysis

To compare TR dynamics among these species, the Kimura two-parameter distance was calculated using the calcDivergenceFromAlign.pl package from RepeatMasker (Tarailo-Graovac and Chen 2009).

### Results

#### Narrow Taxonomic Expansion of TR Transposons

To determine the taxonomic distribution of TR, the S. salar TR transposase sequence was used as a query to perform a TBlastN search in the NCBI whole-genome shotgun database, which contains all of the sequenced genomes from prokar-yotes and eukaryotes. In turn, the newly obtained TR transposases were used as queries to identify additional TR elements. The TBlastN search revealed that this family has a very restricted taxonomic distribution in genomes because it was only present in one superclass (ray-finned fish) and two orders (anura and squamata) of vertebrates. In greater detail, this family exhibited a patchy distribution in vertebrates and was only detected in 85 species of ray-finned fish, 4 species of anura, and 2 species of squamata (table 1). Within the lineage of ray-finned fish, it invaded into 75 species of 22 defined orders and 10 unranked species (table 1 and supplementary table S2, Supplementary Material online). TR also invaded into four amphibian species (Nanorana parkeri, Rana catesbeiana, Rhinella marina, and Xenopus tropicalis) and two species of reptiles (Python bivittatus and Salvator merianae; fig. 2 and table 1). In invertebrates, a similarity with TR was detected in a single flatworm species (Gyrodactylus salaris), in which it encoded a truncated transposase. However, the flanking genomic sequences and the hallmarks (TIRs and TSDs) of Tc1/mariner were undetectable; furthermore, it was identified in a very short contig. Thus, this result seems to have stemmed from sequence contamination and was excluded from further analysis.

We also found that most TR transposons were truncated: in ray-finned fish, more than half of the species (73/85) contained FL TR elements that comprised two detectable TIRs and TSDs; however, only 30 of them contained intact TR copies. Two species of frogs also contained intact copies of TRs (supplementary table S2, Supplementary Material online). The number of TR copies per genome varied significantly across species, ranging from one to several thousands (>80% of identity and >1,000 bp in length) in some ray-finned fish species, such as Oncorhynchus kisutch, Oncorhynchus mykiss, Oncorhynchus tshawytscha, S. salar, Salvelinus alpinus, and Thymallus thymallus, which exhibited 2,420, 3,843, 5,550, 3,259, 2,791, and 3,467 copies, respectively, which suggests...
that these transposons underwent species-specific proliferation in their host genomes. Among anurans, >8,000 copies of TRs were detected in *Rhi. marina*, >2,000 of which were intact. The remaining three species of frogs, that is, *N. parkeri*, *R. catesbeiana*, and *X. tropicalis*, contained 59, 559, and 44 copies of TRs, respectively, but only *X. tropicalis* contained 2 intact copies of the TR. In addition, 255 copies of TRs were detected in one species of python (*P. bivittatus*) and 1 copy of TR was observed in one species of lizard (*Sal. merianae*). The single copy of TR detected in the snake was truncated but retained coding capacity for the transposase (344 aa); however, the TIR and TSD hallmarks were absent. Moreover, all FL TRs detected in lizards were truncated and exhibited loss of coding capacity (supplementary table S2, Supplementary Material online).

Highly Conserved Structural Organization of TRs

The structural organization of TRs was highly conserved across different classes of animals, including fish, frogs, a python, and a lizard. Most of the intact TR transposons had a total length of ~1.5 kb (range 1.0–2.2 kb) and harbored a single open reading frame encoding a transposase of ~340 aa (range 304–350 aa) (fig. 3A). The length variations of transposons are caused by the variable length of the 5′- and 3′-untranslated regions. Several conserved motifs that are characteristic of Tc1/mariner transposons (Plasterk et al. 1999) were also observed in the TR transposase sequence. First, two helix-turn-helix (HTH) motifs were detected at the N-terminal of the transposase and may play a role in DNA binding (Nagy et al. 2004; Rousseau et al. 2004; Feschotte and Pritham 2007); each of these motifs consisted of three alpha-helices. Second, a GRPR motif was detected between the two HTH motifs. Third, an NLS was identified in most transposases that overlapped with the C-terminal of the second HTH motif. Finally, a catalytic triad DDE motif was observed within the catalytic domain, represented by 35 aa located between the second aspartic acid (D) and the glutamic acid (E) (fig. 3B). In addition, all TR elements identified here had short TIRs (13–68 bp) and contained a highly conserved CAGTC (51/78) or CAGCC (23/78) motif at the end of these repeats, which was flanked by canonical 5′-TA-3′ TSDs (supplementary table S2, Supplementary Material online). This differed from the motifs observed in several known DD34E/Tc1 transposons, such as CAGTT in *SB* (Ivics et al. 1997), CAGTG in *Frog Prince* (Csaba et al. 2003), and CAGTG in *Passport* (Clark et al. 2009).

Evidence of the Presence of HTs of TRs and Origin of TRs

The phylogenetic position of TRs was inferred using the maximum likelihood method in the IQ-TREE based on the alignment of the conserved DDE domain (~150 aa). The known families of Tc1/mariner transposons were used as reference families, and transposase 36 in *Rhodopirellula baltica* SH 1 (TP36_RB), which is an insertion sequence of bacteria that is close to the Tc1/mariner superfamily in phylogenetic position (Bao et al. 2009), was used as the outgroup. The access numbers of the reference Tc1/mariner elements are listed in supplementary table S3, Supplementary Material online. The phylogenetic tree showed that DD35E/TR formed a monophyletic clade and was defined as a new family (fig. 4A and supplementary fig. S1, Supplementary Material online); this family was more closely related to DD34E/Tc1 and DD36E/Tc1 than it
| Taxonomic Distribution | Number of Species Containing a TR | Number of Species Containing a FL TR | Number of Species Containing an Intact TR | Length of the FL TR | Length of the Intact TR | Transposable Length of the Intact TR | TR Length of the Intact TR | Number of Copies of the Intact TR | TSD |
|------------------------|----------------------------------|-------------------------------------|------------------------------------------|-------------------|------------------------|-------------------------------|--------------------------|-------------------------------|-----|
| Actinopterygii         | 85                               | 73                                  | 30                                       | 1,072-2,765       | 1,072-2,191            | 303-350                       | 13-68                    | 1-142                         | TA  |
| Beryciformes           | 1                                | 1                                   | —                                        | 1,275             | —                      | —                             | —                        | —                             | TA  |
| Carangiformes          | 6                                | 6                                   | 3                                        | 1,379-1,566       | 1,558-1,566           | 349-350                       | 38-39                    | 1-3                           | TA  |
| Centrarchiformes       | 3                                | 3                                   | 3                                        | 1,072-2,191       | 1,072-2,191           | 311-338                       | 13-52                    | 1-1                           | TA  |
| Characiformes          | 2                                | 2                                   | —                                        | 1,505-1,556       | —                      | —                             | —                        | —                             | TA  |
| Chiliformes            | 9                                | 6                                   | —                                        | 1,520-2,047       | —                      | —                             | —                        | —                             | TA  |
| Oupeliformes           | 2                                | 1                                   | 1                                        | 1,559             | 1,559                  | 337                           | 65                       | 1                             | TA  |
| Cypriniformes          | 8                                | 8                                   | 4                                        | 1,535-1,557       | 1,554-1,557           | 349-349                       | 18-28                    | 3-142                         | TA  |
| Cyprinodontiformes     | 12                               | 12                                  | 7                                        | 1,228-1,579       | 1,461-1,579           | 303-338                       | 16-28                    | 1-3                           | TA  |
| Esoctiformes           | 1                                | 1                                   | —                                        | 1,204             | —                      | —                             | —                        | —                             | TA  |
| Gadiformes             | 4                                | 4                                   | —                                        | 1,202-1,554       | —                      | —                             | —                        | —                             | TA  |
| Gymnotiformes          | 1                                | 1                                   | —                                        | 1,510             | —                      | —                             | —                        | —                             | TA  |
| Lophiiformes           | 1                                | 1                                   | —                                        | 1,534             | —                      | —                             | —                        | —                             | TA  |
| Mugiliformes           | 1                                | 1                                   | 1                                        | 1,552             | 1,552                  | 338                           | 43                       | 1                             | TA  |
| Oscoeliformes          | 2                                | 2                                   | 1                                        | 1,559-1,565       | 1,559                  | 305                           | 38                       | 1                             | TA  |
| Perciformes            | 5                                | 3                                   | 1                                        | 1,217-1,566       | 1,566                  | 338                           | 26                       | 9                             | TA  |
| Polymeliformes         | 1                                | 1                                   | —                                        | 2,531             | —                      | —                             | —                        | —                             | TA  |
| Salmoniformes          | 7                                | 7                                   | 5                                        | 1,548-1,567       | 1,548-1,567           | 319-338                       | 30-68                    | 1-110                         | TA  |
| Scoriformes            | 2                                | 1                                   | —                                        | 1,565             | —                      | —                             | —                        | —                             | TA  |
| Siluriformes           | 3                                | 3                                   | 2                                        | 1,195-1,553       | 1,527-1,553           | 324-349                       | 26-38                    | 1-8                           | TA  |
| Synbranchiformes       | 1                                | 1                                   | —                                        | 1,580             | —                      | —                             | —                        | —                             | TA  |
| Tetraodontiformes      | 3                                | 2                                   | —                                        | 1,125-1,447       | —                      | —                             | —                        | —                             | TA  |
| Unrank                | 10                               | 6                                   | 2                                        | 1,525-2,765       | 1,558-1,566           | 338-338                       | 31-50                    | 2-2                           | TA  |
| Anura                 | 4                                | 4                                   | 2                                        | 1,545-1,944       | 1,563-1,565           | 338-350                       | 26-39                    | 2-300                         | TA  |
| Squamata              | 2                                | 1                                   | —                                        | 1,521             | —                      | —                             | —                        | —                             | TA  |
was to other families of Tc1/mariner, which was confirmed by the phylogenetic tree that was generated using the FL transposases (supplementary fig. S2, Supplementary Material online). To confirm the evolutionary relationship between these families, we also generated a sequence identity matrix using the FL transposases. The matrix indicated that TRs exhibited the highest sequence identity to DD34E/Tc1, followed by DD36E/IC (fig. 4B). Therefore, we assumed that TR evolved from the DD34E/Tc1 family independently from DD36E/IC transposons and formed a separate family within the Tc1/mariner superfamily.

Based on the phylogenetic analysis, the TR elements were classified into four clusters (A–D): cluster A was detected in 26 ray-finned fish species, 2 anura species, and 2 squamata species; clusters B and C were present in 8 ray-finned fish species and 4 ray-finned fish species, respectively; and cluster D was detected in 12 ray-finned fish species and 1 anura species (supplementary fig. S1, Supplementary Material online). The observation that these four clusters, and even the whole TR family, exhibited a discontinuous distribution in animals ruled out the possibility that TR elements were vertically inherited from the last common ancestor of these species. To corroborate this conclusion, pairwise distances between the recombination-activating gene 1 (RAG1) and all consensus sequences or representative sequences of TR were calculated and compared (supplementary table S4, Supplementary Material online). RAG1 is an ideal locus for testing hypotheses about phylogeny and diversification times in vertebrates (Hugall et al. 2007; Gilbert et al. 2012; Zhang et al. 2016). The distances of almost all pairwise comparisons (447/518) were extremely small (0.094 ± 0.056) compared with those calculated for RAG1 (0.255 ± 0.154) (fig. 5A). Almost all TR pairwise distances computed here involved species that diverged from each other >212.8 Ma (supplementary table S4, Supplementary Material online). Given these large divergence times and the absence of purifying selection on TR
sequences, the extremely low pairwise TR distances calculated here seem to be incompatible with a scenario that invokes vertical inheritance of these transposons from the ancestor. Furthermore, in many cases, the sequence identities of TRs were extremely high compared with the divergence time of their hosts (fig. 5B). For example, >83.63% identity was observed between TRs in the fish and frog, which diverged >435 Ma. Similarly, the fish and lizard, which shared the last common ancestor ~435 Ma, showed >80.14% identity (fig. 5B). This value is unexpectedly high considering the deep divergence detected between their hosts. The phylogenetic tree (supplementary fig. S1, Supplementary Material online) and TimeTree (supplementary fig. S3, Supplementary Material online) revealed incongruence between the TR and host phylogeny, which, in combination with the discontinuous
Figure 6.—Evolutionary dynamics of TRs in vertebrates. RepeatMasker utility scripts were used to calculate the K divergence from consensus sequences or the representative sequence (Tarailo-Graovac and Chen 2009). Species with less than ten copies of TRs in their genomes were excluded from the analysis. The y axis represents the coverage (kb) of each TR element in the genome and the x axis indicates the Kimura divergence estimate.
Evidence of Recent Invasions of TRs in Vertebrates

To illustrate further the evolution profiles of TR elements in vertebrates, we compared the evolutionary dynamics of TR elements across species and clusters using a Kimura divergence analysis and sequence identity, the results of which are summarized in figures 5b and 6. The sequence identity matrix showed that the overall average sequence identity (82.33 ± 10.01%) of TRs across species was substantially higher than that reported previously for DD36E/IC (52.48 ± 19.19%) (Sang et al. 2019). Each cluster, including clusters A (83.23 ± 9.38%), B (82.75 ± 10.54%), C (77.62 ± 12.38%), and D (81.50 ± 9.72%), displayed a high-sequence identity between species (fig. 5B), indicating that these four clusters may represent relatively recent HT events. The Kimura divergence estimations of TR elements revealed differential evolutionary dynamics of TRs in vertebrates because most species in cluster A experienced multiple waves of invasion of TRs, whereas most species in the remaining three clusters experienced a single wave amplification of TRs. Moreover, all species in clusters B, C, and D, with the exception of the TR in Cyprinodon variegatus, and some species in cluster A (Amphiphrion ocellaris, Amphiphrion percula, Carassius auratus, Clarias batrachus, Carinus carpio, Epinephelus lanceolatus, Labeo rohita, Simochromis diagramma, and X. tropicalis) exhibited very low Kimura divergences (<5%) (fig. 6), indicating that these species experienced very recent invasions of TRs. These data, combined with the discovery of intact TRs and high-sequence identity in these species, suggest that this family of transposons is a clade of Tc1/mariner that evolved very recently and may still be active in some lineages of animals.

Very Recent Invasions of TRs in Vertebrates

Several lines of evidence from the current study also supported the hypothesis that the DD35E/TR is a family that evolved very recently from the DD34E/TRc1 transposons. First, most other families of Tc1/mariner, such as DD37E/TRT and DD36E/IC, seem to be distributed in both vertebrates and invertebrates, some of which are even very widely distributed, such as DD34E/TRc1 (Vos et al. 1993; Radice et al. 1994; Lam et al. 1996; Ivics et al. 1997; Sinzelle et al. 2005) and DD34D/mariner (Robertson 1993; Plasterk et al. 1999; Arkhipova and Meselson 2005; Nguyen et al. 2014); in contrast, DD35E/TR seemed to exhibit the narrowest taxonomic distribution in animals and was only detected in vertebrates. Second, the average sequence identities of TRs between species across the four clades were very high (>80%), which differed from that observed in other families, such as DD37E/TRT (Zhang et al. 2016) and DD36E/IC (Sang et al. 2019), in which some clades exhibited high identity, whereas others displayed low-sequence identity (Sang et al. 2019). Third, the analysis of the evolutionary dynamics of TRs in these species revealed that most invasions were recent, with a Kimura divergence <5%, which was confirmed by the detection of many intact copies in these species. Taken together, these data indicate that TRs represent very recent invasion events in animals and may still be active in many species.

Discussion

Reorganization of the DD34E/TRc1 Family Based on Conserved Catalytic Motifs

DNA transposons, such as piggyBac, Pelement, hAT, and Tc1/mariner, usually transpose through a cut-and-paste mechanism. They are characterized by the presence of TIRs flanking a gene encoding a transposase that catalyzes the transposition reaction (Hickman and Dyda 2015). Despite the differences in transposition mechanisms, the transposases of some DNA elements are thought to have evolved from a common origin and share similar motifs in their catalytic domain (Capy et al. 1996). The Tc1/mariner superfamily is ubiquitous and forms the largest group of eukaryotic class II TEs. The common motif in these families is a conserved D (Asp) DE (Glu) or DDD catalytic triad, and multiple distinct intrafamilies have been identified to date based on this conserved catalytic domain (Bouuaert et al. 2015; Sang et al. 2019). This study provided the first in silico evidence of a new family (DD35E/TR) of this superfamily of transposons, which displayed the closest phylogenetic relationship and highest sequence identity to the DD34E/TRc1 family, strongly indicating that it may have evolved from this family. This represents the second discovery of a sister family of DD34E/TRc1 in animals, after the initial discovery of DD36E/IC in animals very recently, which also exhibited the closest phylogenetic relationship with the DD34E/TRc1 family and was suggested to have originated from this family (Sang et al. 2019). Our previous study indicated that the DD34E/TRc1 transposons display a high diversity at the family level because at least five distinct clusters or subfamilies (Passport-like, SB-like, Frog Prince-like, Minos-like, and Bari-like) were identified (Gao et al. 2017). In fact, the DD38E transposons identified in sturgeon have also been proposed to be a close sister family of DD34E/TRc1 (Pujolar et al. 2013). These data suggest that the DD34E/TRc1 transposons exhibit an unexpected diversity and may evolve into many families as a common ancestor. The systematic definition of the diversity of the DD34E/TRc1 family in future studies may help illustrate the evolution landscapes of this family, as well as of the Tc1/mariner superfamily.

HT Events of TRs

Horizontal transfer is the transmission of genetic material by means other than parent-to-offspring ones, which is a common occurrence in bacteria (Gogarten and Townsend 2005) but is considered a rare event in eukaryotes (Kidwell 1993;
Andersson 2005). However, a growing body of evidence suggests that the HT of TEs, a particular type of HT, was very common during the evolution of eukaryotes; moreover, it is recognized increasingly as a source of genomic innovation (Wallau et al. 2012; Husnik and McCutcheon 2018). Multiple examples of HT events in vertebrates have been defined well, including diverse DNA transposon superfamilies, such as hAT (Gilbert et al. 2012), PiggyBac (Pagan et al. 2010), Chapaev (Zhang et al. 2014), and Tc1/mariner (Kuraku et al. 2012; Oliveira et al. 2012; Zhang et al. 2016), indicating that DNA transposons play important roles in shaping the evolution of genomes in vertebrates. In addition, our data revealed that the taxonomic distribution of TRs is limited, probably because of the young invasion history of this family in animals or the low promoter activity of TR transposases. Promoter strength has been suggested as a driving force of the transposon HT process (Palazzo et al. 2017). A blurry promoter, which excluded their role as a vector of HT of Tc1 which is defined as a common feature of diverse mariner HT process (Palazzo et al. 2017). A blurry promoter, which is defined as a common feature of diverse Tc1 and mariner elements, including BarI, Sleeping Beauty, and Hsmar1, may play roles in their evolutionary success and is probably involved in overcoming the barriers that exist between the transcriptional machinery of unrelated species (Palazzo et al. 2019). However, this feature is not found in the hobo transposon and LTR retrotransposons (Palazzo et al. 2019) and may be absent in TR elements, which constitutes a barrier to the HT of TRs. Although the mechanism of HT remains unclear, bacteria and pathogens may play a facilitating role, and parasites (such as viruses, ticks, nematodes, and insects), which engage in long-lasting and physical contact with their hosts, may act as shuttles or vectors for the HT of TEs between species (Wallau et al. 2018). The current study provided evidence of the presence of HT events of TR in vertebrates; however, the vectors of the HT events of this family remain unclear. Our data revealed that TR was mainly distributed in ray-finned fish, anura, and squamata, and no parasites of these species, which are potential vectors of HT of TRs in vertebrates, were detected. Although lampreys, which are opportunistic parasitic feeders that attach to large fish using their cup-like mouth to suck their blood and body fluids, were suggested as possible vectors of HT events of DNA transposons in ray-finned fish (Kuraku et al. 2012; Zhang et al. 2014), they were absent from the list of TR invasion species, which excluded their role as a vector of HT of TRs in ray-finned fish. In addition, a Blast search against the Nucleotide Collection (nr/nt) database at the NCBI that was aimed at identifying potential vectors using TR transposases as queries did not identify any TR homology sequences other than those detected previously. Thus, the potential transmission vectors of HTs of TR transposons remain unknown.

**Supplementary Material**

Supplementary data are available at Genome Biology and Evolution online.

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