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Characterization of nonautonomous *Tc1*-like transposable elements of channel catfish (*Ictalurus punctatus*)

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Abstract

Putative nonautonomous transposable elements from channel catfish (*Ictalurus punctatus*) were identified. They were named *Tipnon* elements for *Tc1*-like transposable elements from channel catfish that are nonautonomous. These elements were defined by their terminal repeats that share identity to members of the known *Tc1*/mariner transposon superfamily. They show structural similarities to *Tc1*-like elements, but share little sequence identity beyond the terminal inverted repeats. They do not harbor any amino acid blocks that show similarities to the *Tc1*-like or other transposases and thus may represent truly nonautonomous transposons in channel catfish. They are abundant in the channel catfish genome with a copy number of 32 000, having 500 base pair per copy, this family of nonautonomous transposon-like elements account for 1.6% of the channel catfish genomic DNA. Their high abundance and transposon-like terminal repeats indicate that they may play important roles in gene evolution and in genomic architecture of catfish. Similarity search for potential coding capacity of the *Tipnon* elements revealed that they contain sequence blocks that can potentially encode amino acid blocks similar to the para-type sodium channel proteins in cockroaches or house flies, proteins that function in the central nervous system as voltage-gated sodium transporters. Sequences surrounding the terminal inverted repeats are divergent from those used by the reconstructed *Sleeping Beauty* fish transposase.

Introduction

Transposable elements can be divided into retrotransposons and DNA transposons based on their mode of transposition (Finnegan 1985). Retrotransposons move via RNA intermediates and depend on reverse transcriptase, while DNA-mediated transposons move through DNA intermediates and depend on transposases. They also differ in phylogenetic distribution. Retrotransposons are more common and abundant in vertebrates, whereas DNA transposons are more common in bacteria, plants, and invertebrates such as fruit flies and nematodes. DNA transposons harbor inverted repeats required *in cis* for transposition. Autonomous DNA transposons also harbor a functional transposase gene to encode the transposase enzyme required for transposition *in trans.* In spite of wide range of distribution (Robertson 1993; Radice et al. 1994), DNA transposons were not discovered in vertebrates until recently (Heierhorst et al. 1992; Henikoff 1992). Since the discovery of the first vertebrate DNA transposons in channel catfish by data base analysis (Henikoff 1992), search for an active copy of DNA transposons has been a keen research area. Recently, DNA transposons in vertebrates have been isolated from a number of vertebrate species, but they have been characterized by far the best in teleosts (Heierhorst et al. 1992; Henikoff 1992; Goodier and Davidson 1994; Radice et al. 1994; Izsvak et al. 1995; Ivics et al. 1996; Koga et al. 1996; Lam et al. 1996a). Members of the *Tc1*/mariner superfamily represent most of the DNA transposons discovered in vertebrates. In addition to teleosts, *Tc1/mariner, tiggers,* and other transposon-like elements have been

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discovered in amphibians (Lam et al. 1996b) and human genomes (Oosumi et al. 1995a; Smit and Riggs 1996).

All the characterized DNA transposons from vertebrates to date are defective in their transposase gene because of extensive deletions, insertions, or base substitutions leading to in-frame termination in their transposase genes. These current nonautonomous transposable elements are believed to be once active elements, but were inactivated through a process of vertical inactivation (Lohe et al. 1995). Several lines of evidence indicate that *Tc1*-like transposons were once active. First, their highly conserved structure is similar to currently active transposons in *Drosophila* and *C. elegans*. Second, *Tc1* elements are highly associated with active genes (Henikoff 1992). Remnants of the *Tc1*-like transposons have been detected from various active genes (Liu et al., unpublished) suggesting they once were very active in transposition. Active transposition was suggested in zebrafish (Lam et al. 1996a) although final proof awaits isolation of a functional copy of the *Tzf* transposons. By artificially reversing the process of vertical inactivation using molecular biology, Ivics et al. (1997) were able to reconstruct an active transposon system, *Sleeping Beauty*, by recovering the transposase open reading frame based on the consensus sequences of many mutated forms of *Tc1*-like elements from fish. This progress not only provides evidence for possible vertical inactivation, but also provides a system that can be applied for mutagenesis sequence tagging and for enhancing transgene integration for transgenics research. Transposon tagging in *Drosophila* and *C. elegans* is an important tool for molecular biology and led to identification and cloning of many important genes. Such a technology was lacking in vertebrates until the reconstruction of *Sleeping Beauty* transposase. It would be a great genetic tool if it can be successfully used in other systems such as channel catfish. Understanding of the endogenous inactivated *Tc1*-like transposons is important for adoption of such an innovative approach.

Based on whether they harbor functional transposase gene, DNA transposons can be classified as autonomous and nonautonomous transposons. Autonomous transposons harbor a functional transposase whereas nonautonomous transposons do not. Autonomous DNA transposable elements are often associated with families of nonautonomous elements (Berg and Howe 1989; Oosumi et al. 1996). Strictly speaking, there are two types of nonautonomous transposons: the ones that harbor a defective transposase gene due to deletions, insertions, or in-frame termination, and the ones that do not harbor any sequences similar to transposases. The former type is not truly nonautonomous because they were once active. They represent deleted versions of the parent elements and share sequence identity with the related autonomous forms (Oosumi et al. 1996). The truly nonautonomous transposable elements share only limited sequence similarity to the autonomous forms, often limited to the inverted repeats (Luo et al. 1991; Oosumi et al. 1996). Inverted repeat sequences are very important for DNA transposon mediated transposition (Ivics et al. 1996). Nonautonomous transposable elements with identical or highly similar inverted repeats can be theoretically mobilized by autonomous transposons that provide transposase *in trans*. Because of the relative abundance of the nonautonomous transposable elements, they have the potential for greater contribution to genomic architecture of the hosts (Oosumi et al. 1996).

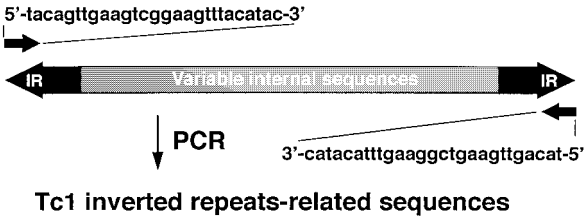

Figure 1. Strategy for amplification of *Tc1*-like elements from channel catfish. PCR reactions were conducted using a single primer of inverted repeat sequences as given.

We previously found co-existence of multiple families of evolutionarily autonomous *Tc1*-like transposons in channel catfish (Liu et al., unpublished). As part of the efforts to characterize the genomic organization and to understand the genomic background of *Tc1*-like transposons in channel catfish, here we report the identification and characterization of a family of nonautonomous *Tc1*-like transposons from channel catfish. They share very limited sequence similarities strictly in the inverted repeat regions to previously reported *Tip1* and *Tip2* (Liu, unpublished) and harbor no sequences similar to transposase, and thus may represent a family of truly nonautonomous transposons in evolution. They are highly abundant in the channel catfish genome and contain sequence blocks similar to the para-type sodium channel proteins from cockroaches (*Blattella germanica*) and house flies (*Musca domestica*).

Materials and methods

Genomic DNA and PCR amplification

Genomic DNA of channel catfish was isolated as previously described using standard protocols (Strauss 1989; Liu et al. 1997, 1998a). Blood was expelled into lysis buffer quickly to disperse the blood cells. The lysates were incubated at 55 °C overnight. DNA was extracted twice with phenol and once with chloroform. DNA was precipitated by adding half the original blood volume of 7.5 M ammonium acetate and two volumes of ethanol. DNA was collected by brief centrifugation and washed twice with 70% ethanol, air-dried, and redissolved in water. The concentration was measured with a spectrophotometer for absorption at 260 nm or estimated from intensities of ethidium bromide staining after agarose gel electrophoresis using 1 kb ladder DNA as reference (GIBCO BRL, MD).

PCR reactions of 50 μl were carried out in 50 mM KCl, 10 mM Tris (pH 9.0 at 25 °C), 0.1% Triton X-100, 0.25 mM each of deoxynucleotide triphosphate (dNTPs), 1.5 mM $MgCl_2$, 20 μM of the primers, about 500 ng channel catfish genomic DNA, and 2.5 units of *Taq* DNA polymerase. The primer used in the PCR represent inverted repeat sequence thus only one primer was used for PCR. Its sequence reads 5′-TACAGTTGAAGTCGGAAGTTTACATAC-3′. The temperature profiles used to amplify the DNAs were 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 2 min, for 35 cycles.

Cloning the putative transposonable elements

PCR products were cloned into pCR2.1 (Invitrogen, CA). Ligations and bacterial transformations were performed with standard procedures (Sambrook et al. 1989). Both the mixture of the PCR products and the gel-purified fragments were used in separate ligations. Mini-scale plasmid preparations were utilized to screen for the clones with PCR product inserts. Clones with expected sizes were picked for sequencing analysis.

DNA sequencing and sequence analysis

All DNA clones were sequenced by the Sanger's dideoxy chain termination method using the cycle sequencing kit (Applied Biosystems, Inc., CA). Minipreparation plasmid DNA (1 μl, about 200–500 ng) was used for each reaction. The profiles for cycling were: 94°C for 1 min, 55°C for 1 min, 72°C for 1 min for 30 cycles. An initial 2 min of extra denaturation at 94 °C were used. Initial sequencing was accomplished with both the universal and the reverse sequencing primers. After initial sequencing reaction, overlapping sequencing was finished by primer-walking method. Sequencing primers were ordered from either National Biosciences, Inc. (Plymouth, MN) or GIBCO (Bethesda, MD). DNA sequences were read and analyzed by using the GCG package (Genetics Computer Group, 1991) or by using a micro-computer software packages DNASIS (Hitachi, version 2.0), or DNASTAR (DNA Star Inc., Madison, WI). Alignments of DNA or protein sequences for identification of homologous sequences were conducted by using BLASTN and BLASTX from NCBI on the internet. Sequences for comparison here were extracted from the non-redundant Genbank+EMBL+DDBJ+PDB databases from the National Center for Biotechnology Information (NCBI) using BLAST searches.

DNA dot blot hybridization

Known amounts of genomic DNA and the plasmid DNA fragment containing the cloned nonautonomous transposon *Tipnon* were first serially diluted. Genomic DNA and *Tipnon* DNA fragment were denatured by mixing with an equal volume of 1 M sodium hydroxide and kept at room temperature for 5 min. An equal volume of 2 M ammonium acetate was added and vortexed to neutralize the samples. The samples were then loaded onto a nylon membrane by using a dot blot apparatus (BRL, Bethesda, MD). One milliliter of 5X SSC was then added to each sample spot. The filter was removed and air dried before baking at 80 °C for two hours prior to hybridization. Hybridization was conducted as described below for Southern blot analysis. The hybridization signals were quantified by using the GS-525 Molecular Imaging System (BioRad, New York) as described (Liu et al. 1998b).

Southern blot analysis

Genomic DNA was amplified with inverted repeat primer (Figure 1) and the PCR products electrophoresed on a 0.8% agarose gel (Southern 1975). The DNA was transferred to a piece of Zetabind nylon membrane (Whatman, Wisconsin) by capillary transfer with 0.4 M NaOH overnight (Reed and Mann 1985). Membranes were dried at 80 °C under vacuum for 2 h. The membrane was washed in 0.5% SDS (w/v) at 65 °C for 15 min and then pre-hybridized in 50%

formamide, 5X SSPE (Sambrook et al. 1989), 0.1% SDS (w/v), 5X Denhardt's and 100 μg/ml sonicated and denatured calf thymus DNA overnight. Hybridizations were conducted overnight at 42 °C in the same solution with probes added (greater than 10^9 cpm per μg DNA). The probes used were fragments isolated from PCR products using primers internal to the inverted repeats sequence used to amplify the transposon-like elements. Probes were prepared using the random primer method (Sambrook et al. 1989) with a labeling kit from Boehringer Mannheim (Indianapolis, Indiana). The Zetabind membranes were washed first in 500 ml of 2X SSC for 10 min, followed by three washes in 0.2X SSC with SDS at 0.2% (w/v) at 65 °C for 15 min each. The membranes were then wrapped by Saran wrap and exposed to Kodak BioMax MS film for two hours.

Results and discussion

Nonautonomous family of Tc1-like transposon sequences

To isolate *Tc1*-elements from channel catfish, genomic DNA was amplified by using a single PCR primer with sequences of the first 27 bp of the zebrafish *Tdr1* (Izsvak et al. 1995) inverted repeats (IR). This PCR reaction allows amplification of elements that share IR sequence identity with those of zebrafish (*Danio rerio*), but have variable internal sequences similar to or different from *Tdr1* (Figure 1). Multiple bands were produced representing multiple families of *Tc1*-related transposon families. We previously identified *Tip1* and *Tip2* families of *Tc1*-like transposons (Liu et al., unpublished). Here we identify and characterize a nonautonomous family of *Tc1*-related sequences and herein we will refer to this family of sequences as *Tipnon* for *Tc1* transposon-related sequences from channel catfish that are nonautonomous.

Nucleotide sequencing analysis revealed that *Tipnon* is a sequence with a size of 535 bp (Figure 2). It has 29 bp inverted repeats that are identical to part of the zebrafish *Tc1*-like element *Tdr1*. However, no indications of any sequence similarities were revealed by BLASTN analysis except at the inverted repeats (Figure 3). To assure that minor sequence similarities may be masked by the high identity in inverted repeats, internal sequences were used alone to search the databases without the inverted repeat sequences. Similar results were obtained without noticeable similarities to any known sequences (not shown). There were even no indications of similar amino acid blocks of transposase in *Tipnon* to any transposase sequences. Therefore, this family of sequences may represent the truly non-autonomous transposons or transposable elements-related sequences. They were defined as *Tc1* transposon-related sequences because their inverted repeats are similar to *Tc1* transposons (Figure 3). The inverted repeat sequences have high identity to the known *Tc1*-like transposon elements from teleost such as *Tss1* and *Tdr1* (Radice et al. 1994; Izsvak et al. 1995). Because the inverted repeats do not code for proteins, this relatedness may be only limited to functional relationships in terms of transposition process. It is known that the inverted repeats are important for transposition by *Tc1*-like transposase (Colloms et al. 1994; Vos and Plasterk 1994; Ivics et al. 1997). Possession of *Tc1*-like inverted repeats may make these elements movable in evolutionary history or may still be movable by transposase recognizing similar inverted repeats *in trans*.

Tipnon is highly abundant in channel catfish genome

Tipnon elements are highly abundant. Dot blot analysis indicated that they exist at approximately 32 000 copies per haploid genome (Figure 4). Given the size of *Tipnon* of 535 bp, it accounts for 1.6% of the channel catfish genome. It differs from other repetitive elements such as the *Xba* elements isolated from channel catfish (Liu et al. 1998b) in that they harbor inverted repeats, characteristic of DNA transposons. The high abundance not only indicates success of this family of elements in molecular evolution, but also suggests a greater potential in shaping genomic architecture of channel catfish by this family of sequences. *Tipnon* is about 8 times more abundant than *Tip2*, and over 200 times more abundant than *Tip1*, both of which harbor similar inverted repeat sequences (Liu et al., unpublished).

Tipnon contains similar sequences to para-type sodium channel gene

Tipnon family of sequences may affect genomic architecture by its cut-and-paste mode of transposition by a transposase from an autonomous *Tc1*-like transposons. It may also influence gene evolution by serving as donor or acceptor of certain amino acid blocks and then shuffles them around in the genome. A BLASTX search using *Tipnon* as a query did not reveal significant similarities to known genes. However, reasonably high similarities between *Tipnon* and the

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TACAGTTGAA GTCGGAAGTT TACATACACC TTGAAGTATC TGCAATATGT TAACATTTTT   60
TTTTTTAAAA AAGGCTTTTT TAATGCTATT TAAAAACTGC TATTTCACAT AACAGATGTT  120
TACATATAGT CCATAAGACA CAATAATAGC TGAATTTACA CAAATGAACC AGTTCAAAAG  180
TTTACACACG CTTGCTTCTT AATACTTTGT GTTGTTACCT GGATGATCAC GATCATGTTT  240
TCATGTTTTG TTAGTCCTTG TTTGCCCAGA GTGGTTAAAA TCCTCAAGTC TGCACTATTA  300
TTTGCTTTAA GCATCTCTAC ATATGTGACC TTTCCTACAG CGGCTATGCG AATGCCAGAG  360
GATCCTCCTT TTCATACTGA GGGCAACTGA GGGACTCGTA CACAACTACA ACATAAAGGT  420
ACAAACATTC ACTGATGCTC AAGAAGGCAC CACACTACAA TAAGAGCCAA ATGGATATAA  480
ACTTTAGAAC AGGATGATGG GTGTAAGCGT ATGTAAACTT CCGACTTCAA CTGTA       535
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Figure 2. Nucleotide sequences of the *Tipnon* element. Inverted repeats are underlined.

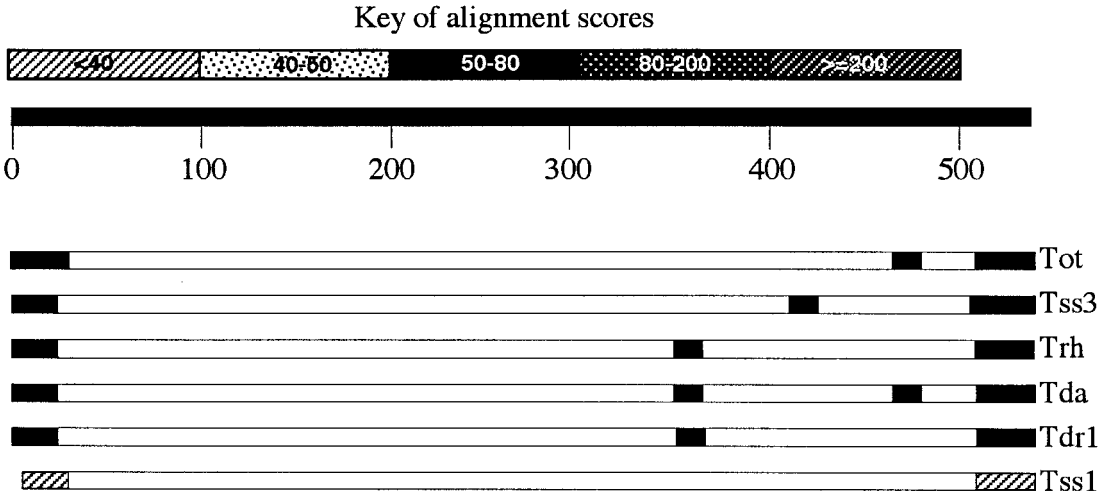

Figure 3. Nucleotide similarities of *Tipnon* elements from channel catfish to other reported *Tc1*-like sequences. *Tipnon* nucleotide sequences were used to search the non-redundant Genbank+EMBL+DDBJ+PDB databases. Alignment scores are raw scores from the BLASTN searches. Open bar indicates no similarity. Accession numbers are as follows: Tot, L41171; Tss3, L12208; Trh, L48684; Tda, L48682; Tdr1, L33471; Tss1, L12206.

para-type sodium channel proteins of cockroaches and house flies were noticed (Figure 5). A 28% identity and 40% similarity exist in a 110 amino acid block, of which two hydrophobic blocks are highly conserved (Figure 5). In the first block of 14 amino acids, 9/14 (64.3%) identity and 11/14 (78.6%) similarity exist. Similarly, in the second block of eight amino acids, 6/8 (75%) identity and 8/8 (100%) similarity exist between *Tipnon* and the para-type sodium channel protein (Miyazaki et al. 1996). Because of the potential transposition activities, *Tipnon* may have been able to shuffle these hydrophobic blocks of peptides around the genome in evolution.

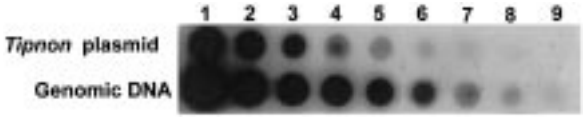

Figure 4. Dot blot analysis of channel catfish genomic DNA (lower row) with *Tipnon* plasmid DNA as controls (upper row) hybridized to the internal segment of *Tipnon*.

Distribution of Tipnon in other fish

To determine the phylogenetic distribution of *Tipnon* elements, genomic DNAs were isolated from channel catfish, blue catfish (*I. furcatus*), white catfish (*Ameiurus catus*), flathead catfish (*Pylodictus olivaris*), carp (*Cyprinus carpio*), tilapia (*Tilapia nilotica*), and pink salmon (*Oncorhynchus gorbuscha*). This PCR ampli-

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Tipnon     1      YS*SRKFTYTLKYLQYVNIFFFKKGFFNAI*KLLFHITDVYI*SIRHNNS*IYTNEPVQK  180
                  Y  S++F+  L YL  + I  F       I  L +H                Y  EP
1633648  1402  YQQSKQFSDVLDYLNMIFIVIFSSECLMKIFALRYH----------------YFKEPWNL  1445

          181   FTHACFLILCVVTWMITIMFSCFVSPCLPRVVKILK--------------SALLFALSIS  318
            F      ++  +   +  I+   FVSP L RVV++ K                  LLFAL++S
         1446  FDFVVVILSILGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKGIRTLLFALAMS  1505
```

Figure 5. Conserved amino acid blocks of *Tipnon* (top line) with the para-type sodium channel protein (bottom line) from the German cockroaches (*Blattella germanica*, accession number 1633648), with identical and conserved amino acid residues in the middle line.

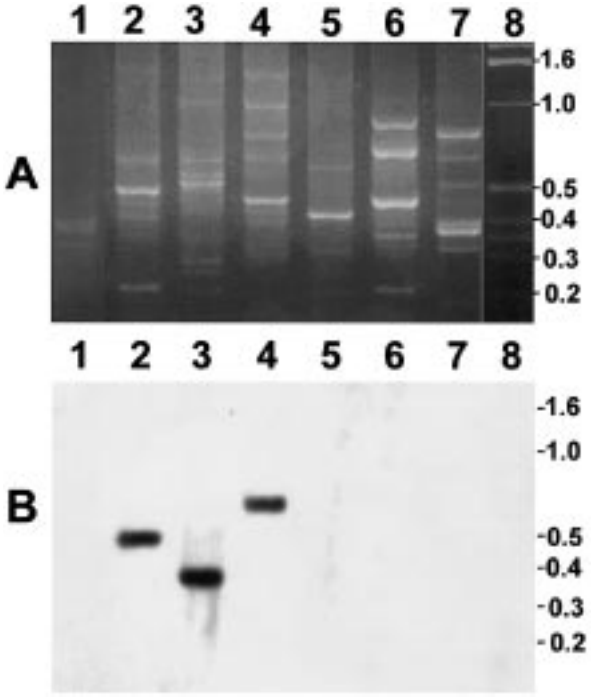

Figure 6. Distribution of *Tipnon* elements in selected teleosts. PCR amplifications were conducted using a single primer of inverted repeats to amplify *Tc1* inverted repeats-related elements. PCR products were separated on 1.0% agarose electrophoresis and stained with ethidium bromide (A), transferred to nylon membrane, and hybridized to the internal segments of *Tipnon* (B). PCR products were amplified from genomic DNA of Atlantic salmon (*Salmo salar*, lane 1), channel catfish (*Ictalurus punctatus*, lane 2), blue catfish (*I. furcatus*, Lane 3), white catfish (*Ameiurus catus* , Lane 4), flathead catfish (*Pylodictus olivaris* , Lane 5), carp (*Cyprinus carpio*, Lane 6), and tilapia (*Orechromis niloticus*, Lane 7). Molecular weight markers in kilo-base pairs (lane 8) are indicated on the right margins of the gel and the blot.

fication produced visible products from all fish tested, indicating existence of sequences with similar inverted repeats (Figure 6A). The amplified products, however, may differ in internal sequences. To demonstrate presence or absence of sequences similar to the internal sequences of *Tipnon*, a Southern blot analysis was performed using the internal sequences without the sequences of inverted repeats as a probe (Figure 6B). *Tipnon* existed in channel catfish, blue catfish, and white catfish, but was absent from all other species tested. This indicates that *Tipnon* is a recently evolved nonautonomous element harboring the inverted repeats similar to those of the autonomous-like*Tip1* and *Tip2* (Liu et al., unpublished).

Evolutionary and practical implications

Identification of *Tipnon* family of nonautonomous elements have important implications. The sequence similarity observed in the inverted repeats of *Tipnon* and other channel catfish *Tc1*-like elements such as *Tip1* and *Tip2* suggest that the mobilization of the small nonautonomous elements is dependent upon the transposase enzyme encoded in the autonomous elements, which may or may not be currently active. Highly conserved terminal repeats would allow use of a single transposase to mobilize a variety of highly divergent sequences in the genome (Federoff 1989).

Identification of *Tipnon* would also facilitate genomic research of channel catfish. It would provide information for data analysis in large scale sequencing programs and for analysis of evolutionary architecture of a given locus harboring any similar sequences to these elements. It accounts for 1.6% of the channel catfish genomic DNA and thus is expected to be encountered frequently. *Tc1*-like elements have been shown to be highly associated with active genes in teleosts (Henikoff 1992; Liu et al., unpublished). They were observed in the introns (Wilson et al. 1990), and adjacent to a number of genes (Mueller-Schmid et al. 1992; Berndtson and Chen 1994; Leaver et al. 1997). In organisms other than fish, transposable elements also have been shown to contribute to coding sequences (Banki et al. 1994), introns (Wessler 1989; Purugganan and Wessler 1992), and regulatory ele-

ments in a wide variety of eukaryotic genes (Stavenhagen and Robins 1988; Chang-Yeh et al. 1991; Dariavach et al. 1991; Bureau and Wessler 1994; Oosumi et al. 1995a, b). Both the diversity of genes in which functional roles can be assigned to transposable elements and the wide phylogenetic distribution of the phenomenon suggest that transposable elements have the potential to play a much greater role than we anticipated in shaping the architecture and establishing eukaryotic gene structure and function in evolution (Oosumi et al. 1996). *Tipnon* elements harbor several hydrophobic domains similar to those in the para-type sodium channel proteins. These hydrophobic blocks could have been building blocks of membrane proteins or their transmembrane domains.

Channel catfish is the most important cultured fish in the United States accounting for over 50% of all aquacultural production. Genetic programs are needed to improve brood stocks through genetic engineering, marker-assisted selection (MAS), selective breeding, or combination of these approaches. Understanding genomic organization would facilitate efforts of gene mapping programs which generate linkage information useful for MAS. We previously identified and characterized the *Xba* family of repetitive elements from channel catfish accounting for 5–6% of the channel catfish genome (Liu et al. 1998b). Here we report the *Tipnon* family of non-autonomous transposons accounting for 1.6% of the channel catfish genome. *Tipnon* differs from *Xba* elements in that it harbors inverted repeats similar to *Tc1*-like transposons and theoretically could be a potential target for transposase actions. Upon transposition, it has the ability to move throughout the genome and can have great effect on genomic architecture.

The sequences around the *Tipnon* inverted repeats are divergent from those of the *Sleeping Beauty* system, suggesting that the endogenous *Tipnon* inverted repeats probably do not compete for binding of the reconstructed transposase, or at least inefficiently if at all, because of the sequence divergence in the inverted repeats which are important for binding (Ivics et al. 1997).

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