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# Expression of DNA transposable elements during nervous system development

## A discussion about its possible functions

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**Transposable elements (retrotransposons and DNA transposons) comprise a large proportion of animal genomes, for example 20% in *D. melanogaster*, 36% in *X. tropicalis* and 45% in humans. After invading a new genome, the transposable element increases its copy number and subsequently accumulates mutations. These may eventually result in inactive copies. Until recent days transposons have been considered "junk" DNA and no clear function has been assigned for this important amount of information on genomes.**

**Due to the possible detrimental effects transpositions can have on genomes, hosts have developed different mechanisms to silence active transposons, including small-interfering RNAs (siRNAs) and PIWI-interacting RNAs (piRNAs). The latter are very often derived from transposable elements and can abolish transposon expression at both transcriptional and post-transcriptional levels.[1,2] Despite these mechanisms, the regulated and localized expression of transposable elements has been reported in several species, suggesting other possible functions.**

**Significant examples are reports on differential expression of retrotransposon families in Drosophila and mouse embryos.[3–5] In a screen for differentially expressed genes in the dorsoventral axis during early *Xenopus tropicalis* development, we found a DNA transposable element, from the *Tc*1 family (*Tc1–2_Xt*), expressed specifically in the dorsal region, which eventually forms the nervous system.[6] Further analyses of this transposable element showed that both strands are expressed in the prospective nervous system. Its transcript is not fused to any other endogenous gene, and piRNAs are derived from this element.[7] Interestingly, we did not find this element in *Xenopus laevis*, a closely related species. However, we did find a very similar expression pattern for two other transposable elements of the *Tc*1 family (*TXr* and *TXz)* in *X. laevis*. Here, we discuss these results as well as recent evidence suggesting that transposons can also have a positive role in host genomes. We propose that transposable elements can play a role in the formation of the nervous system and discuss possible experimental strategies to investigate this hypothesis.**

**Keywords:** transposon, Tc1, regulated expression, Xenopus

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## Expression of Transposable Elements from the *Tc1/Mariner* Family during Early Development

The regulated expression of different families of retrotransposons and DNA transposons has been described in different tissues and species. However, a detailed analysis of the transcripts or their temporal and spatial expression patterns has been reported in only a few cases. During Drosophila embryogenesis, the differential temporal expression of retrotransposons has been characterized.[3] Spatial differential expression of the retrotransposon *412* has been described, specifically in the gonadal mesoderm.[4] In mouse, retrotransposons make a high contribution to the pool of

maternal mRNAs in early embryos, and the expression of these elements is developmentally regulated.[5] In Xenopus, the *1A11* retrotransposon-like element is specifically expressed in the mesoderm and its expression is regulated by FGF.[6] Likewise, the expression of the retrotransposon family *Xretpos* is restricted to ventro-posterior regions during development.[7]

We extended these observations by showing that a DNA transposable element of the *Tc*1/*mariner* family is differentially expressed during *X. tropicalis* development.[8,9] The general structure of *Tc*1/*mariner* elements is shown in **Figure 1A**. Transcripts for some *Tc*1-like elements have been detected in other EST databases,[10-16] but no detailed analyses has been performed. Our studies showed that the *Tc*1-like element *Tc1–2_Xt* mRNA (named according to the RepeatMasker nomenclature) is specifically expressed at the gastrula stage in the Spemann's organizer. This region is required for the proper dorso-ventral and anterior-posterior patterning of the embryo and the neuroectoderm, the tissue that will give rise to the nervous system (**Fig. 1B**). Then as development progresses, *Tc1–2_Xt* mRNA is found restricted to neural tissue regions. Both *Tc1–2_Xt* sense and antisense strands present similar expression patterns. The length of the mRNAs suggests that these transcripts are not included in other genes. Transcripts from *Tc1–2_Xt* are detected mainly after zygotic transcription has begun. piRNAs are specifically derived from *Tc1–2_Xt*, and analysis of the expression of selected *Tc1–2_Xt*-derived piRNAs suggests that these elements control its temporal expression.

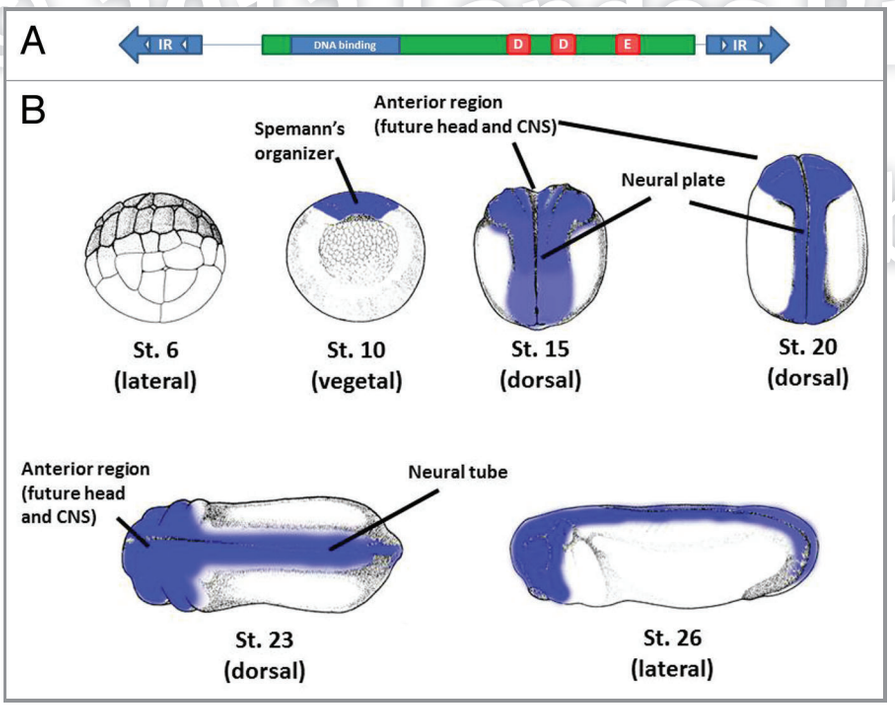


**Figure 1.** Putative structure of *Tc1–2_Xt* transposable element and expression during development. (A) The sequence analysis of the 116 copies of the *Tc1–2_Xt* transposable element in the *X. tropicalis* genome showed the typical structure of *Tc*1-like elements. The length of this element is 1,581 bases. It is flanked by two inverted repeats (IR) containing two direct repeats (white triangles) for the binding of the transposase. The transposase ORF (green) contains a putative DNA binding domain (blue) and the catalytic triad (red, aspartic-aspartic-glutamic residues (DDE)). Five copies in the genome could code for an intact transposase. (B) Schematic representation of the expression of *Tc1–2_Xt* during *X. tropicalis* early development. The expression of *Tc1–2_Xt* is shown in blue for early stages of *X. tropicalis* development. No expression is detected before the beggining of the zygotic transcription (st.6 is shown as an example, lateral view). Expression is clearly detected from gastrula stage (st.10) in the Spemann's organizer and then in anterior and neural tissues. Views are indicated in brackets. CNS, central nervous system. Figures were downloaded from http://www.xenbase.org/anatomy/static/NF/NF-all.jsp and modified according to the expression pattern obtained by in situ hybridization.

In the *X. tropicalis* genome, 72% of transposable elements correspond to DNA transposons[17] with seven families of *Tc*1-like elements characterized.[18] Our analyses for these elements, albeit not detailed, have shown that several of these families are transcribed during *X. tropicalis* development.[9]

Interestingly, we could not find *Tc1–2_Xt* in *X. laevis* by using RT-PCR or in situ hybridization. Whether this is explained because this element invaded the *X. tropicalis* genome after both species diverged, or to a high divergence in the sequence in the *X. laevis* genome is not known. The availability of the *X. laevis* genome could be very useful to study these alternatives. However, in *X. laevis*, two *Tc*1-like elements have been described in the genome, *TXr* and *TXz*.[19] Our studies have demonstrated that *TXr* and *TXz* are also expressed during *X. laevis* development. Importantly, the expression pattern of these elements is very similar to *Tc1–2_Xt* in *X. tropicalis* and both strands are also expressed.

At present, we do not know how the expression of these elements is controlled and whether one or several loci are being transcribed. The presence of regulatory elements controlling gene expression in the sequences of these *Tc*1-like elements has not been studied. The expression can be regulated by endogenous promoters controlling neural-specific genes. However, in contrast to the expression of *Tc*1 elements in *C. elegans*, which occurs by fortuitous read-through transcription,[15] most *Tc1–2_Xt* RNA is not included in other protein-coding transcripts, for which read-through transcription is unlikely to explain the bulk of *Tc1–2_Xt* RNAs. Another alternative is the presence of clusters of several copies of transposable elements under the control of a single promoter. The transcription of clusters of transposable elements to generate piRNAs has been described in Drosophila.[20] It is possible that these clusters can also be regulated during development to produce specific expression patterns. Another explanation is that transcription is ubiquitous, and that *Tc1–2_Xt* RNAs are degraded in ventral and posterior regions

by a piRNA dependent-mechanism. In any case, the specific temporal and spatial expression of all these elements strongly suggests that they could play a role during nervous system development.

## Possible Roles of Transposable Elements as Non-Coding RNAs

In the *X. tropicalis* genome, 111 out of 116 *Tc1–2_Xt* copies contain frame-shifts and mutations rendering putative transposase-inactive copies of the element. Consistently, the analysis of 20 *Tc1–2_Xt* cDNAs from gastrula stage embryos indicates that none of them codes for an active transposase. These results suggest that *Tc1–2_Xt* could play a role as a non-coding RNA. In mouse, the transcription of the retrotransposon SINE B2 is necessary for gene activation in the growth hormone locus.[21] In this case, the expression of this retrotransposon regulates the formation of a euchromatin/heterochromatin boundary, resulting in the expression of genes in proximal regions. It is still unknown whether this is the case for *Tc1–2_Xt* expression, but we can propose that some of the *Tc1–2_Xt* loci may be regulating chromatin architecture, allowing the expression of genes involved in the formation of the nervous system.

Considering that piRNAs derived from *Tc1–2_Xt* were detected, another possible function is to generate piRNAs. The expression of both strands is consistent with the ping-pong model of amplification of piRNAs.[20,22] If this is the case, it is worth noting that the *Tc1–2_Xt* RNA is stable enough to be detected by RT-PCT, RNA gel blot and in situ hybridization, suggesting that the piRNAs produced are most likely not enough to degrade all of the *Tc1–2_Xt* RNA during early development. In addition, the expression of sense and antisense strands is specifically detected in neural tissues, and therefore, the amplification of piRNAs could occur in these tissues. Alternatively, piRNAs derived from *Tc1–2_Xt* may not be involved in the degradation of *Tc1–2_Xt* itself. Rather than that, they may regulate endogenous genes that contain sites complementary to piRNAs. Although our analysis for two *Tc1–2_Xt*-derived piRNAs showed that all the piRNA sequences in the genome are included only in *Tc1–2_Xt* sequences, the possibility that other *Tc1–2_Xt*-derived piRNAs map to genes cannot be ruled out. For example, piRNAs derived from transposable elements regulate the expression of endogenous genes and allow the clearance of maternal mRNAs during early Drosophila development.[23] Finally, recently it has been published that piRNAs derived from non-repetitive regions have a role in spine morphogenesis in the central nervous system in mice.[24] Therefore, *Tc1–2_Xt* -derived piRNAs may have a role in Xenopus neural physiology. Similar scenarios can be proposed for *TXr* and *TXz* in *X. laevis*.

## Possible Roles of Transposable Elements as Active Copies

Although all the *Tc1–2_Xt* cDNA copies we analyzed do not contain a functional transposase open reading frame, 5 out of 116 *Tc1–2_Xt* complete sequences in the genome could putatively code for an open reading frame containing the catalytic triad and perhaps an active transposase (**Fig. 1A**). We don't know if these five copies contain all the other residues required for transposition, such us the binding to DNA domains. However, the presence of active copies in the genome and its possible expression cannot be ruled out.

Interestingly, work from the Gage laboratory showed that endogenous LINE-1 retrotransposition can occur during mouse development.[25] In addition, LINE-1 retrotransposition in the vicinity of neural genes can alter the expression of these genes in neural precursor cells. Furthermore, the same group showed that LINE-1 retrotranspositions can also occur in neural progenitor cells isolated from human fetal brain, suggesting that de novo LINE-1 retrotransposition events may occur in the human brain.[26] These events produce mosaicism in the neurons due to different genomic modifications on different neurons in the same individual. This has been suggested as a novel mechanism involved in the generation of the astonishing neuronal diversity required for nervous system formation.[25-27] Therefore, the specific expression of *Tc1–2_Xt* (and *TXr* and *TXz*) in dorsal and neural tissues allows speculation about a similar role in Xenopus. As active elements for transposition, these transposons could be involved in the generation of heterogeneity during Xenopus nervous system development.

## Experimental Approaches to Study the Role of Transposable Elements during Development

In this section we will briefly discuss possible experimental approaches to evaluate transposition of *Tc*1 elements during nervous system development and determine its possible contribution to produce neuronal diversity.

One of the first questions is to determine whether expression of an active DNA transposase occurs during early development. After cloning the possible candidates, transposase activity must first be determined by in vitro assays.[28] For this, a plasmid reporter for excision events needs to be prepared. Based upon the *white-peach* allele studied in Drosophila,[29] we devised the plasmidial DNA excision reporter construct shown in **Figure 2**. The *egfp* open reading frame contains an insertion of a random sequence (same length as the transposase) in between of the inverted repeats the putative transposase recognizes. Therefore, when co-expressed with the transposase mRNA in an exogenous system (e.g., cell culture), successful translation into an active transposase would render EGFP+ cells. Excision footprints would have to be devised in frame with the *egfp* gene.

Experiments to demonstrate in vivo transposition could be performed. For this purpose, transgenic *Xenopus* embryos containing the reporter construct in **Figure 2** can be obtained.[30] A tissue-specific promoter would allow us to follow transposition in the central nervous system. The presence of an endogenously active transposase would render EGFP+ cells that we could observe in later developmental stages, such as stage 50 tadpoles. Furthermore, it would be possible to characterize transposon insertion sites in neural tissues of individual tadpoles using deep sequencing of reverse PCR amplicons (see **Fig. 2**). The comparison of insertion sites in neural tissues with

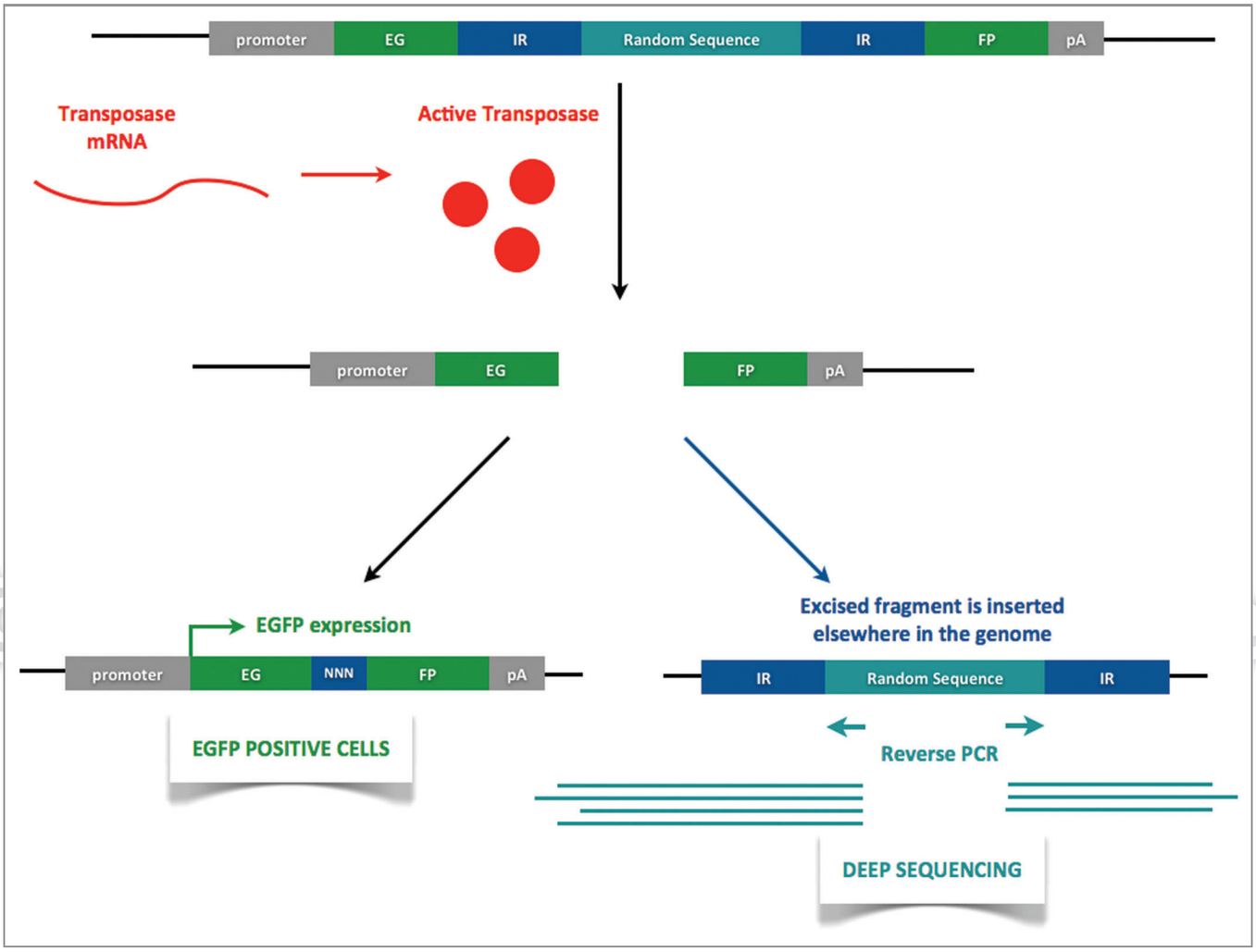


**Figure 2.** Transposition reporter construct. The reporter construct contains an *egfp* open reading frame with an insertion of a random sequence (same length as the original transposase). When transposase mRNA is present (either exogenous, when working in vitro, or endogenous, when in vivo) and an active transposase is being translated, transposition will occur, rendering EGFP+ cells. Note that transposition footprint is in frame with the *egfp* gene. The random sequence insertion sites can then be characterized using deep sequencing of reverse PCR amplicons. IR- inverted repeat. EGFP-enhanced green fluorescent protein. pA-polyadenylation signal. NNN-transposition footprint.

non-neural tissues could be an indicator of in vivo transposition events.

All of these approaches can be useful to determine whether transposition events occur during Xenopus development. Although host cells contain mechanisms to avoid the expression of transposable elements, they could also be using these elements for cellular functions. A proper balance must exist to control these positive and negative effects of transposable elements. Our work has shown that the specific expression of *Tc*1 elements in neural tissues and suggests that transposable elements may play a role during the formation of the nervous system in vertebrates.


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