Review

The DNA transposon *Minos* as a tool for transgenesis and functional genomic analysis in vertebrates and invertebrates

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Abstract

Transposons are powerful tools for conducting genetic manipulation and functional studies in organisms that are of scientific, economic, or medical interest. *Minos*, a member of the *Tc1/mariner* family of DNA transposons, exhibits a low insertional bias and transposes with high frequency in vertebrates and invertebrates. Its use as a tool for transgenesis and genome analysis of rather different animal species is described.

Introduction

Transposons employ intricate mechanisms of excision and reinsertion, and they appear to play an important role in the evolution of their hosts, often accounting for a substantial part of the genomes [1-5]. This makes transposons in themselves interesting biologic systems that are worth studying. More recently, however, they have primarily attracted attention as tools for the manipulation of the genomes of their natural hosts and, more importantly, the genomes of other species that are not closely related. Transposons have even been found to be active in organisms that do not belong to the kingdom of their natural host [6,7]. This review summarizes the advances that have been made with one of these versatile genomic tools, namely the transposon *Minos*.

History of *Minos*

Minos is a DNA (class II) transposon that was fortuitously identified as a repetitive element in the genome of the fruit fly *Drosophila hydei*. The natural element, approximately 1.8 kilobases in length, is characterized by 254 base pair (bp) perfect inverted terminal repeats flanking a two-exon transposase gene (Figure 1). Sequence comparisons revealed homology with mobile elements of the *Tc1/mariner* superfamily of transposable elements [8]. The *Minos* element carries a single gene that is interrupted by a 60 bp long intron and encodes a transposase. The amino-terminus of the transposase contains a putative DNA-binding domain resembling the paired domain, a conserved feature of the Pax protein family [9]. The carboxyl-terminus of the *Minos* transposase contains a D,D34E catalytic triad, which is also found in transposases of related elements such as *Tc1* and *Bari1* [10]. The presence of an unmapped nuclear localization signal has been inferred based on the nuclear localization of a *Minos* transposase-enhanced green fluorescent protein (EGFP) fusion [11].

It was demonstrated that the *Minos* element actively transposes in the *D. hydei* germline [9]. Surveys among *Drosophila* spp. have revealed widespread occurrence of *Minos*-like elements; 21 out of 26 analyzed species of the repleta group and 5 out of 7 analyzed species of the saltans group carry *Minos*-like transposons. Evolutionary analysis suggests that the distribution of *Minos* in the genus *Drosophila* is best explained by horizontal transfer of the element across species [12,13].

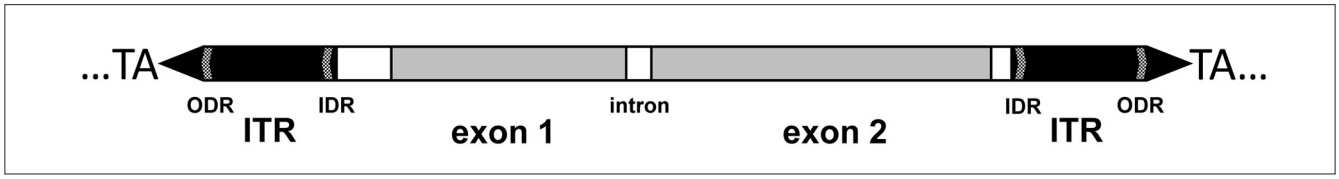

Figure 1
Structure of the natural *Minos* element isolated from *Drosophila hydei*. The transposase gene is interrupted by a 60 base pair long intron. Not all features are drawn to scale. IDR, inner direct repeat; ITR, inverted terminal repeat; ODR, outer direct repeat.

The mechanism of transposition

Like most DNA transposons, *Minos* moves in a host genome with a cut-and-paste mechanism, whereby the transposase excises the element from the original site of insertion and reinserts it into a new locus in a nonreplicative manner. The transposition of *Minos*, like that of the other *Tc1/mariner*-like elements [10], occurs into a TA dinucleotide that is duplicated upon insertion [9]; this implies that a staggered cut of the target DNA leads to 2 bp single strand TA overhangs as the first step in the insertion reaction. Analysis of the sequences that flank insertion sites in the genome of *Drosophila melanogaster* revealed that *Minos* transposase has little sequence preference beyond the TA target dinucleotide [14]. This is in contrast to most other transposable elements studied thus far, which exhibit variable degrees of flanking sequence preference, resulting in biased insertion and consequently the existence of 'hot' and 'cold' spots along the genome [15,16].

Although much of the recent work on transposons is concerned with the transgenesis of vertebrates, simpler model organisms and cell lines are more suitable for the analysis of the transposition mechanism. The introduction of a non-autonomous *Minos* element and of a transgene expressing transposase into the *D. melanogaster* genome [17] allowed the study of the *Minos* transposition mechanism by mobilization of the non-autonomous element and subsequent molecular analysis of the excision and transposition events [18]. Two types of chromosomal sites were recovered after excision: sites precisely restored and sites containing leftovers (footprints) of the mobilized element. Precise excision (restoration of the original site of insertion) was detected only in flies heterozygous for the insertion, presumably resulting from gene conversion [18]. Most footprints were 6 bp long and consisted of the four terminal nucleotides of either one of the inverted repeats plus the duplicated TA dinucleotide. The structure of the footprints suggested a possible mechanism of excision involving staggered cuts by the transposase at the ends of the inverted repeats, creating single strand overhangs of the four terminal bases of each end. During reinsertion, the overhangs of the mobilized element are joined to the TA overhangs of the target site, followed by polymerase-mediated fill-in of the single stranded DNA. This leads to regeneration of the transposon ends and duplication of the target TA. This model predicts that a heteroduplex flanked by the TA duplication is formed at the site of excision. Indeed, the existence of such heteroduplexes in the genome of *D. melanogaster* flies in which *Minos* is actively excising was directly demonstrated [18].

Several excision sites were also cloned from vertebrate cells, in which a single non-autonomous *Minos* element was mobilized by *trans* acting transposase. These footprints, however, were much more heterogeneous in their size and structure, reflecting differences in the post-excision processes compared with *Drosophila* [19].

An analysis of *Minos* excisions from a plasmid in the simple chordate *Ciona intestinalis* identified the typical 6 bp footprints as well as variants thereof with short insertions or deletions [11].

Sequence-specific interaction of transposase and the transposable element at or near the inverted repeats is required for transposition. Each of the *Minos* inverted repeats contains two 18 bp direct repeats near the ends of the inverted repeat (Figure 1). DNAaseI protection with purified recombinant *Minos* transposase protein shows that these direct repeats are bound by transposase (Figure 2a).

Although the binding site close to the end of the element (the 'outer' direct repeat) is expected to play a role in the excision of the element, the function of the second, 'inner' repeat is not known. In addition to *Minos*, two other well studied transposons of the *Tc1/mariner* family carry two direct repeats per inverted terminal repeat: *Tc3* and *Sleeping Beauty*. Transposon mobility assays suggest that both the outer and the inner pairs of binding sites are required for full activity of *Sleeping Beauty* [20,21] and *Minos* (Klinakis A and Savakis C, unpublished data) but not that of *Tc3* [22]. The mechanism underlying the involvement of the inner direct repeat in *Minos* and *Sleeping Beauty* transposition remains to be elucidated.

It is intriguing that the inner direct repeat overlaps a putative TATA box in the 5' inverted repeat of *Minos*, and primer extension analysis revealed a transcriptional initiation site for the *Minos* transposase approximately 30 bp downstream of this repeat (Figure 2b). These observations

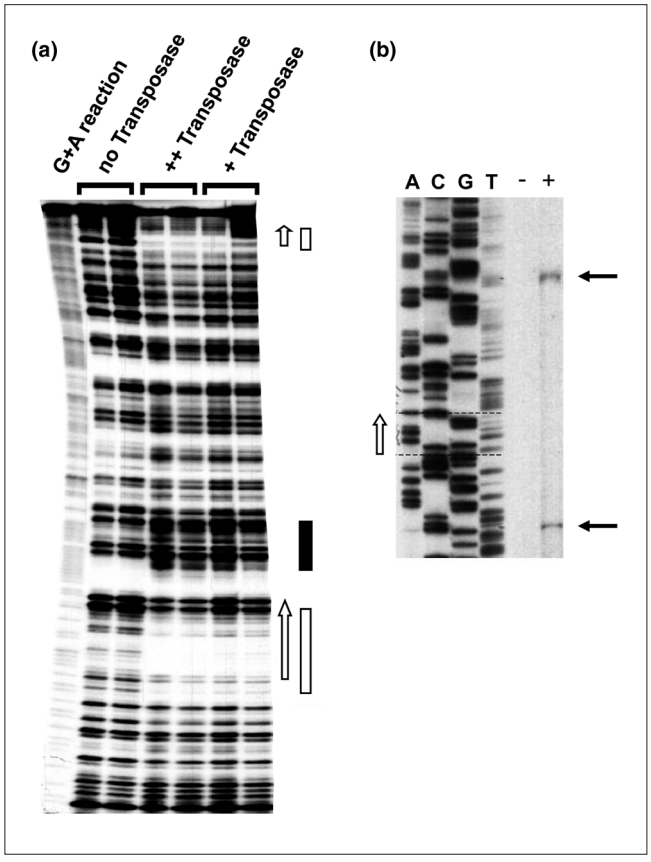

Figure 2
Binding of transposase to the *Minos* inverted terminal repeat and internal *Minos* transcription initiation sites. **(a)** DNAasel footprint of a *Minos* inverted terminal repeat. 'G+A reaction' indicates G and A specific sequence reaction; the DNAasel protection reactions were done in duplicate. '+ transposase' indicates twofold molar excess of transposase over target DNA, and '++ transposase' indicates 20-fold molar excess of transposase. The positions of the protected sequences (open boxes), the hypersensitive positions (filled box), as well as the positions of the inner (bottom arrow) and outer direct repeats (top arrow) are indicated. Experimental details are available on request. **(b)** Promoter activity of a *Minos* inverted terminal repeat. 'A', 'C', 'G', and 'T' indicate base specific sequencing reactions. Also shown are primer extension reactions with a *Minos*-specific primer on total RNA isolated from *D. melanogaster* populations with (+) or without (-) a *Minos* insertion. The lower horizontal arrow indicates a transcription start about 30 base pairs downstream from the inner direct repeat. An additional transcription start site is detected between the two direct repeats (upper horizontal arrow). The vertical arrow and dashed lines indicate the inner direct repeat.

suggest a possible role for the inner transposase binding site in autoregulation of transposase expression. Negative feedback mechanisms that regulate transposition have been suggested for other transposable elements too [23-25].

In vitro activity assays established for other members of the *Tc1/mariner* transposon superfamily have demonstrated that no specific host factors are essential for transposition [26,27]. Although direct biochemical evidence is not yet available for *Minos*, the ability of *Minos*-based vectors to transpose in cells of a wide range of metazoan species [19,28-40] is indicative of the element's host independence (Figure 3).

Minos as a tool for genetics

Transposons can be used as vectors for genetic manipulation. The inverted terminal repeats are the only *cis*-acting sequences necessary for transposition. Transposon mediated transgenesis mimics nature; many transposons found in genomes are non-autonomous derivatives. Autonomous elements have intact inverted repeats and carry an active transposase gene. Non-autonomous elements also carry functional inverted repeats but no functional transposase gene, because of the accumulation of point mutations and deletions. Elements that lack an intact transposase gene are unable to catalyze their own transposition, but they can be mobilized with transposase supplied in *trans*. In a typical engineered transposon, the transposase gene is inactivated through the insertion of a selective marker or deleted and replaced by a marker gene. For transgenesis, the transposon is injected into early embryos. Transposase is supplied in *trans* from a copy of the transposase gene introduced into the genome, or (more often) expressed from co-injected plasmid or transposase mRNA. The transient nature of the supply of transposase is the key to the generation of stable insertions. A construct carrying a non-autonomous transposon is usually termed a 'donor' and a construct carrying an active transposase gene is termed a 'helper'.

The *Minos* transgenesis system is supported by an extensive toolkit that has allowed experimentation in a wide spectrum of species. First, extra-chromosomal assays have been devised to facilitate and speed up the process of assessing the effectiveness of the *Minos* components in diverse organisms of interest [11,28,33,34,36,41]. Second, an ever-increasing number of *Minos*-based transformation vectors has been created, containing either fluorescent proteins such as GFP or more traditional marker genes for the easy identification of integration events and transgenic individuals [14,17,30-32,35-37,39,40,42-44]. Third, the availability of different transposase sources, varying from a chromosomal copy of the transposase gene to a helper plasmid or *in vitro* synthesized mRNA, can fit most purposes and experimental settings [17,28,32,35,45].

The development of *Minos*-based gene manipulation for mammals was based on a first phase of evaluation in insects. As in the case of all other insect transposons, *D. melanogaster* has been the favored testing ground for most components and applications of the *Minos* system. The original use of *Minos* as a germline transformation vector in *D. melanogaster* [17] was followed by the transformation of the medfly *Ceratitis capitata*, in the first report of a transposon system applied in a genus other than the one in which it was originally identified [32]. The validation of

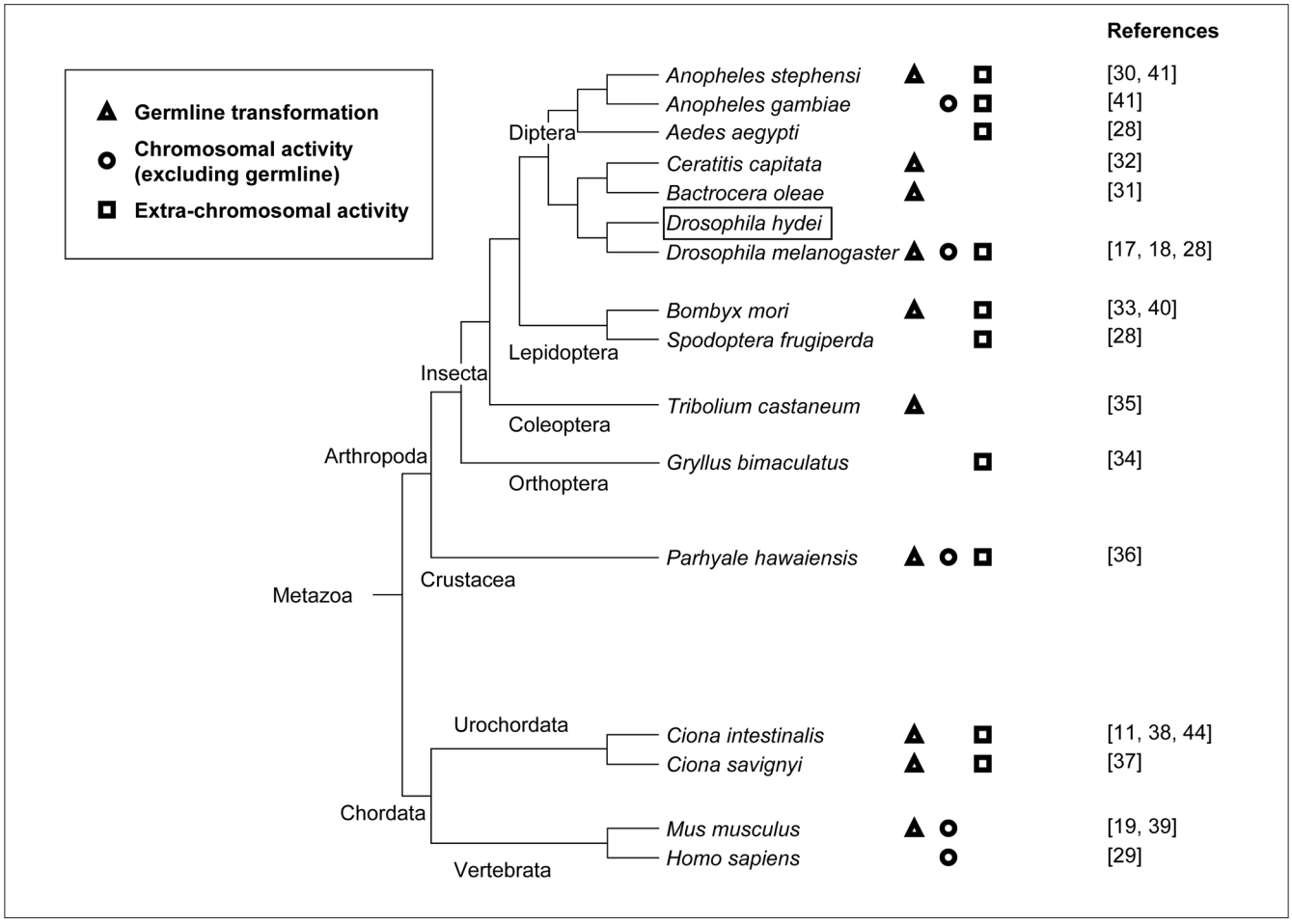

Figure 3
Simplified Cladogram of species in which *Minos* has shown to be active. The original host (*Drosophila hydei*) is boxed. Assays for three types of *Minos* activity are distinguished and indicated (extrachromosomal, chromosomal, and germline).

improved *Minos* tools in activity assays conducted in mosquito and lepidopteran embryos and cell lines [28,33, 34,41] opened the way to the stable germline transformation of a species of great medical importance, namely the human malaria vector *Anopheles stephensi* [30]. More recently, the development of even more efficient *Minos* transposase sources [45] and of versatile fluorescent marker systems [31,46,47] facilitated the genetic transformation of diverse arthropod species, ranging from Diptera (the olive fly *Bactrocera oleae* [31]) to Coleoptera (the red flour beetle *Tribolium castaneum* [35]), Lepidoptera (the silkworm *Bombyx mori* [40]), and Crustacea (the amphipod *Parhyale hawaiensis* [36]).

The genetic transformation of *D. melanogaster* using the P-element over 25 years ago [48,49] triggered the development of innovative experimental approaches that revolutionized molecular genetic analysis in this model organism. However, the use of the P-element is limited to species closely related to *D. melanogaster*. The establishment of *Minos* and other transposon systems allowed a number of these forward and reverse genetic methodologies to be transferred to several non-Drosophilid insects of experimental and practical interest, as well as to a Crustacean. For instance, analysis of *cis* regulatory elements with reporter constructs has been demonstrated in *Ceratitis* and *Parhyale* [36,50], enhancer trapping has been undertaken in *Tribolium* [35] and *Parhyale* (Pavlopoulos A, Averof M, unpublished data), conditional mis-expression systems have been developed in *Anopheles* and *Parhyale* [43] (Pavlopoulos A, Averof M, unpublished data), and post-transcriptional gene silencing via RNA interference constructs has been employed in *Anopheles* [51].

Minos activity is not limited to arthropod species; it has also been used for germline transformation and enhancer trapping in two species of ascidians [11,37,38,44,52]. Finally, *Minos*-based transposons have been mobilized successfully in cultured human cells and in the mouse soma and germline [19,29,39].

In addition to *Minos*, there is a handful of other DNA transposons used for vertebrate transgenesis, the most widely employed of which is *Sleeping Beauty*. The relative evaluation of these different transposons is difficult unless experiments are performed under comparable conditions, which is generally not the case. However, one such comparison has been done with regard to transposition efficiency of the transposons *Sleeping Beauty*, *Tol2*, *Mos1*, and *piggyBac*. In all four different vertebrate cell lines tested, *piggyBac* clearly outperformed the other elements [53]. An analogous direct comparison of these elements with *Minos* has not yet been performed. One should, however, be careful not to take the transposition rate as the only criterion of the usefulness of a transposable element as a genomic tool. Depending on the intended application, insertional preference, the capacity to carry large inserts, mutagenicity, the ability to create deletions upon excision, and other properties can be at least equally important as high transposition frequency.

Minos as a tool for genomics

The availability of sequenced genomes for several model and nonmodel organisms signals the next challenge in genomics research, namely the functional annotation of all predicted genes. Classic genetic analysis relies on the use of mutations to study the function of the affected gene. The most common experimental approach is inactivation of the gene by chemical mutagenesis or insertional mutagenesis using transposable elements or viruses. Insertional mutagenesis has the advantage that the targeted gene is at the same time tagged with the inserted DNA, and so it is relatively easy to identify and retrieve. In principle, the short target sites of most transposable elements would allow a single transposon to saturate the whole genome with mutagenic insertions. In practice, however, every transposable element exhibits insertional biases for certain genomic loci, rendering mutagenesis of the entire genome problematic. For example, the ability of the P-element to target new genes in *D. melanogaster* appears to have reached its limits [16,54]. In accordance with the distinct target preferences between *Minos* and P, a pilot study [14] indicated that more than half of *Minos* generated insertions were in genes not previously hit by the P-element.

Although the P-element tends to insert in 5' untranslated regions, *Minos* insertions in *Drosophila* genes occur preferentially into introns. These insertions appear to be spliced out with the targeted introns and thus do not interfere with normal gene function. However, re-mobilizing such insertions by providing transposase *in trans* leads at reasonable frequency to deletions of flanking DNA, so that induced excision can be used to 'knockout' the targeted gene [14]. Because of the ability of *Minos* to transpose at high frequencies and the fact that 60% of all *Minos* insertions were in or close to genes, the *Minos* system has become an integral component of the *Drosophila* Gene Disruption Project, which is a systematic effort to mutate all genes in the *Drosophila* genome [55] (Bellen H, personal communication).

The efficiency of large-scale mutagenesis screens is enhanced in genetic schemes that utilize two different transposons, say A and B. The 'jump starter' element A (with inverted repeats A) encodes transposase B that, when expressed, mobilizes the 'mutator' element B (with inverted repeats B) into new genomic loci. The independent development of both *Minos* and *piggyBac* based gene transfer systems in the beetle *Tribolium castaneum* [35,46] allowed the initiation of a large-scale insertional mutagenesis screen by the GEKU consortium (research groups based in Goettingen, Erlangen, Kansas, and the US Department of Agriculture Grain Marketing and Production Research Center). In such screens, *Minos* might be more useful as the mutator element because, unlike *piggyBac*, it can excise imprecisely, offering the possibility to generate mutant alleles secondarily.

Other transposon based approaches to genomic analysis include the various 'trapping' schemes, in which expression of a reporter or selection gene carried by the inserted transposon depends on the targeted gene. In enhancer trapping [56] the expression of a transposon encoded marker gene is activated or modulated by transcription enhancer elements near to the transposon insertion site. In such traps, expression of the marker gene follows partly or fully the spatial and temporal expression of the gene normally controlled by the 'trapped' enhancer elements. *Minos* based enhancer trapping has been demonstrated in the ascidian *Ciona* [57], in *Tribolium* [35], in *Parhyale* (Pavlopoulos A, Averof M, unpublished), and in *D. melanogaster* (Metaxakis T, Savakis C, unpublished data).

In exon trapping, expression of a transposon-encoded marker gene depends on appropriate splicing of the marker gene upon insertion of the element into an intron [56]. The preference of *Minos* to insert into introns in the fly and the mouse [14] (Zagoraiou L, unpublished data) suggests that *Minos* may be particularly useful for this type of analysis. Indeed, the choice of an exon trapping *Minos* based transposon as mutagen in human cells in culture was justified by the outcome of the experiment [29]. This transposon carried an intronic splice acceptor followed by a selection gene that would be expressed only upon insertion into an intron of an active gene and then spliced in the correct frame to the upstream exon. Using this transposon mediated mutagenesis (TRAMM) method, it was demonstrated that the *Minos* system can potentially trap/tag all genes in a mammalian genome. Co-transfection of a *Minos* transposon construct with a helper plasmid expressing transposase into HeLa cells resulted in 4% of the cells being stably transformed, with on average two *Minos* insertions per transformed cell.

In later studies, efficient inter-chromosomal transposition of *Minos* was demonstrated in double transgenic mice, carrying tandem copies of non-autonomous *Minos* elements and the *Minos* transposase gene under the control of tissue specific promoters. The observed transposition rate was much greater in oocytes than in lymphocytes (8% versus 0.6%) [19,39]. Its high germline activity makes *Minos* a powerful tool for genome-wide functional studies in whole mice and probably in other vertebrates.

Conclusion

Minos has attracted interest as a tool for basic research, for biotechnological purposes [58], and for developing strategies to control arthropod disease vectors and agricultural pests [30-32,51]. *Minos* has been successfully evaluated as a tool for the transgenesis of diverse animal species, both vertebrates and invertebrates, whereas its functionality in plants has yet to be tested. In most species tested, *Minos* transposition occurs with high frequency, transposons can carry relatively large transgenes, insertions are stable in the absence of transposase [58], and the only obvious target site preference is the TA dinucleotide. Overall, these features demonstrate the usefulness of *Minos* as a tool for functional genetic and genomic approaches in model and nonmodel organisms, including vertebrates.

Competing interests

CS is a founding member and nonexecutive director of a company, Minos BioSystems Ltd (http://www.minosbiosystems.com), which owns intellectual property related to the Minos transposable element.

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