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 Bar1 [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].
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, confirming the existence of ‘hot’ and ‘cold’ spots along the genome [15,16].

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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 (Tc3 and Sleeping Beauty) 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

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**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.
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
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, Most1, 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 invertebrates.

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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