Review

Transposon mediated transgenesis in a marine invertebrate chordate: *Ciona intestinalis*

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**Abstract**

Achievement of transposon mediated germline transgenesis in a basal chordate, *Ciona intestinalis*, is discussed. A *Tc1/mariner* superfamily transposon, Minos, has excision and transposition activities in *Ciona*. Minos enables the creation of stable transgenic lines, enhancer detection, and insertional mutagenesis.

**Introduction**

DNA transposons are powerful tools for genetic analyses. Transposons are employed for creation of stable transgenic lines, enhancer detection, gene trapping, and insertional mutagenesis. These transposon-mediated techniques have been facilitated by the discovery and reconstruction of active transposons in several organisms [1-11]. Despite their utility, transposon technologies are restricted to a few model organisms. Marine invertebrates include most of the phyla whose study is crucial to elucidating the evolutionary molecular mechanisms of diversification in metazoans. To date, transposon technologies have been introduced for only a few marine invertebrate species. Because of the scarcity of refined genetic techniques, research into gene functions in marine invertebrates has remained limited.

Recent achievement of germline transgenesis in a marine invertebrate chordate, *Ciona intestinalis*, has altered this situation [12,13]. *C. intestinalis* has several characteristics that make it amenable for genetics research. In this basal chordate, a *Tc1/mariner* superfamily transposon, Minos, has the complete activity required for its transposition [14]. Minos introduced into *Ciona* is excised from a plasmid vector by transposase and is integrated into TA dinucleotides of another DNA molecule. The TA dinucleotides are known as target sequences of *Tc1/mariner* transposons [15]. Transposition occurs in *Ciona* germ cells, and Minos is inserted into the chromosomes of germ cells [12]. The insertions are inherited stably by subsequent generations, thereby creating stable transgenic lines. Using this transformation technique, genetic techniques such as enhancer detection and insertional mutagenesis have been introduced into *Ciona* using Minos [13,16,17]. In this article, recent achievements with transposon techniques in *Ciona*, as well as characteristics of *Ciona* as a new genetic model, are discussed.

**Characteristics of Ciona intestinalis as an experimental system for genetics**

Ascidians, or sea squirts, are members of the subphylum Tunicata [18]. Tunicata belong to the phylum Chordata along with Cephalochordata (amphioxus) and Vertebrata (Figure 1a) [19,20] (Kawashima T, Putnam N, personal communication). As this phylogenetic position suggests, ascidians possess a simplified chordate body plan. This characteristic is most apparent in their larval stage. The larvae of ascidians are typical tadpole larvae and swim like fish (Figure 1b). Each larva has a dorsal hollow neural tube and notochord, both of which represent common characteristics of chordates. In contrast to those apparent similarities, the
ascidian larval body is strikingly simple compared with that of vertebrate tadpoles. The typical ascidian larva consists of numerous, but countable, cells [18]. For example, the larvae of C. intestinalis consist of approximately 2,600 cells, of which 40 constitute the notochord, 26 make up the muscle, and about 330 the nervous system [18,21]. Ascidians develop rapidly; many ascidians complete embryogenesis within 1 day. This simplicity and rapid embryogenesis aid detailed cell-by-cell analyses of the mechanisms of tadpole body formation. In fact, ascidians are the only chordates for which cell lineages have been described [22-24].

Figures 1
An ascidian - Ciona intestinalis. (a) Phylogenetic relationships of chordates. Ascidians are included in the subphylum Tunicata. (b) A Ciona intestinalis larva. This photograph was constructed by merging three photographs of the same individual. Scale bar: 100 μm. (c) Ciona intestinalis adults. After metamorphosis, Ciona loses its tail and starts to settle. Most ascidians are filter feeders.

Surgically collected eggs can be fertilized with sperm from a different individual. They subsequently exhibit normal, synchronized development. Natural spawning can be induced by simple dark-light adjustment to facilitate self-fertilization. Moreover, sperm can be stored on ice for 1 week without loss of fertility. Cryopreservation of sperm is also established to store mutants or transgenic lines semi-permanently [34]. The easy handling of germ cells enables reduction in labor associated with mutant screening and preservation of lines.

The most striking characteristic that distinguishes Ciona from other ascidians is the availability of a draft genome sequence [35]. The Ciona genome size is approximately 166 megabases per haploid, which contains 15,852 protein coding genes [36]. The genome size and gene number are comparable to those of Drosophila melanogaster, and much smaller than those of most vertebrates. In addition, the Ciona genome is less redundant than those of vertebrates [37,38], which is probably related to the twofold to threefold duplication of genomes during vertebrate evolution [39]. Therefore, Ciona possesses the basic set of genes for a chordate body plan. Because of its compact genome, Ciona provides a simple experimental system in which to uncover genetic mechanisms that specify the chordate body plan as well as mechanisms of chordate evolution.

An unusual aspect of the Ciona genome with respect to transposons is that an extensive search of the Ciona draft genome identified no Tc1/mariner transposon (Table 1). Taking into consideration the global conservation of this transposon family [40], the absence of Tc1/mariner transposon in Ciona genome is curious. Two possibilities are readily apparent. One is that Tc1/mariner transposon has been lost in the Ciona, ascidians, or tunicates branch by accumulation of mutations. Another possibility is that a hypothetical suppressor of this transposon family interfered with the lateral transfer of transposons. This might be related to the weak transposon activity of several Tc1/mariner transposons in Ciona, as discussed below.

In Ciona, techniques to support the practice of genetics research have been developed. The Ciona life cycle is about 2 to 3 months. An inland culture system has been established [17,34]. Settlement after metamorphosis enables retention of several lines in the same aquarium. Introduction of DNA and RNA into eggs by microinjection or electroporation is performed routinely [41,42]. The latter technique can introduce DNA and RNA into hundreds of eggs within 1 hour, thereby facilitating creation of transgenic lines.

There are three major obstacles to use of Ciona to conduct genetics studies. First, no inbred strain has been created; most experiments are dependent on natural populations. Creating strains had been difficult because of complications in culturing. Recent improvements of inland culture systems

Figure 1
An ascidian - Ciona intestinalis. (a) Phylogenetic relationships of chordates. Ascidians are included in the subphylum Tunicata. (b) A Ciona intestinalis larva. This photograph was constructed by merging three photographs of the same individual. Scale bar: 100 μm. (c) Ciona intestinalis adults. After metamorphosis, Ciona loses its tail and starts to settle. Most ascidians are filter feeders.
are expected to resolve this problem [43]. Second, natural
Ciona harbor many single nucleotide polymorphisms. The
genome project reported that 1.2% of nucleotide differences
were observed between alleles of the single individual [35].
This score is 15 times higher than that in humans, and three
times higher than that in pufferfish. Such highly frequent
polymorphism would render it difficult to perform system-
atic fine mapping of point mutations. On the contrary,
high polymorphism might allow retention of highly frequent
natural mutants, which are a valuable resource for mutant
screening. In C. intestinalis, and its related species
C. savignyi, several mutants have been isolated through
screening of wild populations [34,44,45]. The third obstacle
to genetics studies is the requirement for seawater for
culture. Large-scale culturing requires a considerable
amount of seawater, which limits the culturing of Ciona to
laboratories that are near to the sea. Recently, Ciona culture
with artificial seawater has been achieved [34,43], which will
promote the spread of Ciona studies to inland laboratories.

Activity of Minos transposon in Ciona
Minos is a member of the Tc1/mariner superfam-
ily of transposons isolated from Drosophila hydei [5]. Minos exhibited
both excision and transposition activity from protostomes to
deuterostomes [46-54], suggesting a wide host range. Minos
is the only transposon whose activity has been described in
Ciona [12-14,55,56]. Its excision is observed in almost all
embryos when Minos is injected into Ciona embryos
together with transposase mRNA (Figure 2). Footprint
sequences indicate that Minos is excised correctly by
transposase. The typical footprint sequences of Minos are 5’-
TACTCGTA-3’ or 5’-TACGAGTA-3’; both typical and atypical
footprint sequences are observed in Ciona [14,57]. The
atypical footprint sequences might be related to the endogenous
repair system of Ciona. Neither excision nor
transposition occurs without transposases, suggesting that
no Ciona protein mimics Minos activity. Interplasmid trans-
position assay using donor and recipient plasmids (Figure 2)
has revealed that Minos has slightly lower transposition
activity in Ciona than in insects [14,49]. The manner of
insertion of Minos into the recipient plasmid is identical to
that previously reported; the target sequences are TA
dinucleotides and the duplication of the TA sequences
occurs, which flanks two inverted repeats. The frequency
of excision and transposition activity suggests that Minos has
sufficient activity to cause germline transgenesis in Ciona,
as shown by microinjection of transposase mRNA with
recombinant Minos containing a promoter-green fluorescent
protein (GFP) cassette. The scheme of screening transgenic
lines is shown in Figure 3. About 30% to 36% of Minos-
jected Ciona become founders and transmit Minos
insertions to progeny. The average insertion number
inherited from a founder was estimated at around two
(Sasakura Y, unpublished data). This transgenesis frequency
is comparable to that of Sleeping Beauty (SB) in zebrafish
[58]. Thermal asymmetric interlaced (TAIL)-polymerase
chain reaction (PCR) is used to identify Minos insertion sites
[12,59]. Minos was preferably inserted into TA-rich
sequences such as introns [12].

Another convenient transgenesis technique of Ciona with
Minos was achieved using electroporation [56]. As described
above, electroporation enables rapid and reproducible trans-
genesis of early Ciona embryos. This technique simulta-
aneously electroporates Minos DNA and in vitro synthe-
sized Minos transposase mRNA in Ciona embryos. The
transformation frequency by electroporation mediated
transgenesis is about 20% to 30%, which is lower than that
by microinjection mediated transgenesis, perhaps because of
a lower amount of mRNA introduced into embryos by
electroporation. By microinjection, 5 to 10 ng/μl of Minos
DNA and 50 to 200 ng/μl of transposase mRNA are included
in the injected solution. The current electroporation method
requires 60 μg of Minos DNA and 60 μg of transposase
mRNA, which would correspond to 5 to 10 ng/μl of DNA and
RNA in the injection solution. Nevertheless, electroporation mediated transgenesis is now the main strategy of Ciona transformation because of its convenience.

Minos exhibited constant excision and transformation activity, even when the length of insertion is sufficiently long to suppress transposition of another Tc1/mariner transposon, namely SB [60]. So far, an insert size of up to 10 kilobases has been found to have no adverse effect on insertion efficiency (Sasakura Y, unpublished data). Such flexibility of Minos with respect to insert length allows the creation of various transposon constructs that are appropriate for experimental purposes.

**Activity of other Tc1/mariner transposons in Ciona**

The identification of other active transposons would make transposon technology more versatile in Ciona, because it would be useful to create ‘jump starter’ lines of Minos. Modifier screens of mutants generated by Minos must be done using a different transposon. Different transposons can be expected to have different insertion site preferences. Therefore, execution of large-scale mutagenesis with two transposons would be effective for saturation mutagenesis. In addition to these technical innovations, description of activity of transposons in various organisms is necessary to elucidate cross-species activity of transposons and the mechanisms that determine transposon activity in nonhost organisms. Such knowledge would be valuable for further improvement of transposon technologies. Transposon activity in marine invertebrates has not been described, except for Minos in Ciona and in a crustacean [12,53]. Ciona is the pioneer organism of transposon technology among marine invertebrates; testing of various transposons in this organism is important.

The Tc1/mariner superfamily includes many transposons whose consistent activity in several protostomes and vertebrates has been described [61-64]. We have tested some of these transposons, including SB, Frog Prince (FP), and Most, in Ciona. The former two transposons are resurrected...
transposons that are derived from vertebrate genomes [8,11]; Mos1 was isolated from the insect Drosophila mauritiana [3,4]. All three transposons are active in vertebrates [11,61,63,64]. Excision activity of the three transposons was examined using a PCR-based assay (Figure 2a), and SB, FP, and Mos1 exhibited excision in Ciona (Figure 4). Their excisions have been supported by the presence of footprint sequences (Figure 4). However, the excision efficiency of these transposons was lower than that of Minos. When the excision efficiencies of transposons were compared with the same condition (5 to 10 ng/μl of transposon DNA and 50 to 200 ng/μl of transposase mRNA in the injection solution), Minos showed excision in almost all embryos, whereas Most, SB, and FP showed excision in only a few embryos. For example, nine out of 16 embryos exhibited excision in the case of Most, two out of 16 in the case of SB, and eight out of 32 in the case of FP. Interplasmid transposition assay and germline transformation of Ciona with Most, SB, and FP were also tested, but no transposition was detected (Awazu S, Sasakura Y, unpublished data).

What might restrict Mos1, SB, and FP activity in Ciona? One possibility is that co-factors that are required for transposase activity are incompatible or absent in Ciona. In fact, SB and FP are transposons derived from vertebrates [8,11], and therefore they retain high activity in vertebrates, indicating that all sets of co-factors required for SB and FP activity are present in vertebrates. Recent studies have revealed necessary co-factors for SB transposases [65,66]. Although Ciona contains the basic set of genes for the chordate body plan, many genes are specific to vertebrates. The supply of such co-factors may be necessary to make transposons active in Ciona if SB and FP transposases require such vertebrate-specific co-factors. An alternative possibility is that a factor is present that inhibits transposases. Inhibition of the transposase activity has been reported in the Tn5 transposon of Escherichia coli, in which an inhibitor of the transposition protein (a truncated form of Tn5 transposase that does not possess DNA-binding activity) forms a complex with Tn5 transposase and interferes with transposition [67]. The presence of such an inhibitor has not been demonstrated in Tc1/mariner transposons, but the possibility remains that there is a Ciona protein that binds transposases and inhibits their activity.

The inefficiency of Most, SB and FP in Ciona implies that activity of transposons must be tested in each animal model to seek an active transposon. Identification of factors that restrict the activity of transposons is necessary to make them more valuable tools for genetics research in various organisms.

**Enhancer detection**

The compact genome of Ciona is a convenient feature for studying regulatory elements of gene expression [68,69]. High density of enhancer elements is expected in the Ciona genome, facilitating efficient enhancer detection, which is necessary to identify enhancers that cannot be identified using conventional cis element analyses. This technique is also useful in creating marker lines that express reporter genes in a tissue-specific manner. In Ciona, techniques of germline transgenesis were established recently, but to date only a few marker transgenic lines are available. Enhancer
promoter for enhancer detection in all tissues. There might be enhancers to which Ci-TPO promoter could not respond, because minimal promoters exhibit different responsiveness to enhancers (Lemaire P, personal communication). In fact, most enhancer detection lines with Ci-TPO promoter showed reporter gene expression in endodermal tissues [70]. Comparing the efficiency of enhancer detection between the Ci-TPO promoter and a basal promoter or a minimal promoter derived from a housekeeping gene may be necessary to identify an ideal promoter for enhancer detection in Ciona.

In the Ci-musashi enhancer detection line, Minos was inserted into an intron [16], Detailed analysis of the line revealed that expression of Ci-musashi is regulated by many enhancers located at the 5' upstream region and in introns [16]. These enhancers have both redundant and distinct functions for gene expression. Such an enhancer complex is probably necessary to ensure the appropriate spatial and temporal expression of Ci-musashi. Enhancer identification in the context of chromosomes is necessary to understand the in vivo function of these enhancers. Enhancer detection is a viable method for this purpose.

Remobilization of Minos in Ciona genome

Non-autonomous transposons in the genome can be remobilized by providing transposase mRNA (Figure 5). This technique is useful for creating new insertions and ‘local hopping’, and for creating new mutant alleles by deletions. Remobilization of Minos within the Ciona genome was achieved by microinjection of transposase mRNA into embryos whose respective genomes contain tandem arrays of Minos (Figure 5a [70]). This method has been used for enhancer detection [70]. We created a transgenic ‘mutator’ line harboring a tandem array of Minos vector for enhancer detection, which contains a promoter of Ci-TPO [70]. The tandem array in the mutator line was estimated to include as many as 255 transposons. In this study, remobilization of a few copies of Minos copies probably occurred from the concatemer. Screened enhancer detection using the remobilization technique was conducted as follows. Transposase mRNA was injected into unfertilized wild-type eggs. These eggs were fertilized with sperm from the mutator line. Because our enhancer detection vector shows GFP expression in a part of somatic cells, these transposase-introduced Ciona were selected to remain as GFP positive, transposon containing animals. These GFP positive animals were crossed with wild-type individuals; then, the GFP expression pattern in the next generation was monitored to screen families exhibiting altered GFP expression.

The results indicated that 79% of transposase-injected animals transmitted enhancer detection insertions (Figure 5b). This frequency is considerably higher than that seen in the microinjection mediated approach. Although many of the

Figure 4
Excision activity of Mos1, SB, and FP transposons in Ciona. (a) (top part) Excision of Mos1. The left panel shows the polymerase chain reaction result of Mos1 transposon and transposase-injected embryos; the right panel shows the results of Mos1 transposon-injected control embryos. The expected sizes of correct excision events are shown by arrowheads. (bottom part) Footprint sequences of Mos1 observed in Ciona and typical footprint sequences reported previously (typical footprint). (b) Excision of Sleeping Beauty (SB). Note that three to six times more transposon DNA was injected in this experiment, which resulted in the detection of excised bands from every embryo. (c) Excision of Frog Prince (FP). bp, base pairs; M, marker lane.
enhancer detection lines showed GFP expression in endo-
dermal tissues, a few lines showed expression in ectodermal or mesodermal tissues. Therefore, this method could be more efficient for large-scale enhancer detection with creation of many valuable lines. The tandem array interferes with detailed analyses of insertions by Southern blot and TAIL-PCR. In fact, Southern blot was done to show the presence of novel insertions created by remobilization. However, the signal was not conspicuous in many individuals, and as a result the number of new insertions is likely to have been underestimated. Identification of new insertion sites by TAIL-PCR was performed after digestion of genomic DNA with restriction enzymes to suppress PCR amplification within the concatemer [73]. Numerous lines have insertion sites that were unidentifiable, even after restriction enzyme treatment. The enhancer detection insertions can be segregated from the original tandem array by passing through several generations. This may result in the establishment of transgenic lines that have a single insertion of enhancer detection in their genome. Characterization of their insertion sites may increase the efficiency of identification of the causal insertions that were obtained using the remobilization technique.

Remobilization of a single Minos insertion might reduce these problems (Figure 5c). Several tests of remobilization of a single insertion have been carried out using microinjection of transposase mRNA into embryos of transgenic lines (Sasakura Y, unpublished data). In somatic cells excision events were observed (Figure 5d). However, the frequency of excision appeared to be low, and evidence of excision or transposition in the germ cells was not obtained. The primordial germ cells of ascidians are suggested to be two small cells, called B7.6, in early embryogenesis [74]. Thus, germ cells are derived from a small number of primordial cells. Less injected transposase mRNA would be delivered to germ cells than to somatic cells. Therefore, the frequency of excision and transposition in the germ cells would be much lower than in the somatic cells. A technical innovation, such as generation of ‘jump starter’ lines, is necessary to achieve highly frequent jumping of a single Minos copy in germ cells [75].

**Insertional mutagenesis**

Insertions of Minos can disrupt gene function to create mutants. Insertional mutants are distinguishable from background mutations by the fact that they segregate with the insertions. In *Ciona*, a small-scale mutagenesis screen was carried out using self-fertilization (Figure 3), and two insertional mutants were isolated from 120 transgenic lines, which are estimated to correspond to 240 insertions; one mutant can be isolated for every 120 insertions. The mutant frequency is lower than with insertional mutagenesis with pseudotyped retrovirus in zebrafish (one mutant per 85
insertions [76,77]). Taking into consideration the compact genome of Ciona, which has less redundancy, it is curious that insertional mutagenesis in Ciona would be less efficient than in zebrafish. There are two possible explanations. One is that the preference of the insertion sites in the gene, such as 5’ end, introns, exons, or 3’ end, might be different between Minos and pseudotyped retrovirus. In the zebrafish approach, approximately 60% of the mutagenic insertions reside in the promoter, first exon, or first intron [77]. As mentioned above, Minos is preferably inserted into TA-rich sequences such as introns and intergenic regions. The second possible explanation is that pseudotyped retrovirus would be more mutagenic than Minos. Introduction of a gene trap cassette into the pseudotyped retrovirus vector did not affect the mutation frequency [77,78]. Pseudotyped retrovirus might interfere with splicing to produce truncated proteins, even without such a cassette. In contrast, the single Minos insertions into introns appeared to be insufficient to cause mutations (Sasakura Y, Awazu S, unpublished data). The mutant frequency would therefore reflect the difference between two vectors.

From mutant screening, one insertional mutant has been characterized in detail [17]. In this mutant, an insertion at the promoter of a gene encoding cellulose synthase (Ci-CesA [79]) disrupts expression of this gene. Animals homozygous for this insertion exhibit abnormalities in the process of metamorphosis. At the larval stage, their trunks show post-metamorphosed states, although they retain tails, which would normally be lost during metamorphosis. The trunk-metamorphosed larvae continue to swim vigorously. This mutant was named swimming juvenile (sj). This mutant showed a novel function of animal cellulose synthase for the process of normal metamorphosis as well as for the biosynthesis of cellulose. As described above, a concatamer of Minos is inserted into the promoter of Ci-CesA. In another insertional mutant (Matsuoka T, Sasakura Y, unpublished data), a concatamer of Minos is inserted into an intron. Such concatamers are very long and may therefore disrupt promoters or introns. However, mutations by a single insertion are superior to concatamers; some refinement of transposon vectors, such as gene trap, is necessary to produce highly mutagenic Minos. Recently, we attempted to introduce a gene trap method into Ciona (Oogai Y, Sasakura Y, unpublished data).

Because insertional mutagenesis with Minos has been achieved, the next step will be saturation mutagenesis using this transposon. Ciona contains a smaller set of genes with less redundancy than in vertebrates. This characteristic renders this ascidian a suitable organism for saturation mutagenesis. It is necessary to estimate the frequency of essential genes for development in order to calculate the number of transgenic lines that are necessary for saturation mutagenesis. For such estimation, isolation of more mutants is necessary. In addition, several obstacles to Ciona genetics must be overcome in order to conduct saturation mutagenesis. One is the need to create a mutagenic Minos construct. Other obstacles are associated with the primitive state of Ciona genetics, resulting from its short history. Although we take only Ciona into consideration here, most of these points also pertain to other marine invertebrates.

In the mutant screen, we used a Minos construct with a GFP reporter. The expression of GFP was used to judge whether mutations are associated with insertions. However, a correlation between a mutation and GFP expression does not always indicate that the mutation is caused by a Minos insertion. A wild population of Ciona was used to create insertional mutants. Wild populations maintain frequent background mutations. Sometimes these natural mutation sites are located very close to Minos insertion sites, and therefore natural mutants, so-called associated mutants, appear to be related to the Minos insertions. These associated mutants must be discriminated from insertional mutants because transposon insertion sites in the associated mutants are close, but not identical, to the actual sites of mutations. In the recent small-scale mutagenesis studies [13], four mutants exhibited strong correlation with GFP expression (>90% of homozygous mutants showed GFP expression). Two of them were associated mutants and two were insertional mutants. Associated mutants are distinguishable from insertional mutants by imperfect correlation between mutations and GFP expression. Reporter gene expression is a good marker for this purpose, because hundreds of mutants can be examined through simple observation. The mutants showing perfect correlation with GFP expression are candidates for insertional mutants. Several experiments must be performed to conclude that they actually are insertional mutants. Identification of the insertion sites is primarily required. It is also necessary to demonstrate perfect homozygosity of mutants with respect to the insertions, which is evidence that recessive insertional mutants have been created. Finally, to establish a causal link, it is necessary to identify those genes that are responsible for mutants; this may be achieved through rescue experiments or knockdown of genes by microinjection of antisense morpholino oligonucleotides or dominant negative forms [17,42].

The second disadvantage of Ciona, after its suboptimal mutation frequency, is that its embryos sometimes develop poorly compared with those of other model organisms. Typical unhealthy development includes kinked tails at the larval stage. Families showing such unhealthy development are omitted from screens. Such omission might cause the loss of mutants that would show the kinky-tail phenotype. Several insertional mutants might have been lost through this technical limitation. Therefore, the mutation frequency with Minos described above might have been underestimated. Recent improvements in culturing systems will enable continual production of healthy embryos. If a family
shows unhealthy development in this setting, then the phenotype is probably derived from mutations.

Forward genetics is a powerful technique in which to identify gene function; it is possible to identify gene functions that are neglected by reverse genetics. This approach has recently been employed in Ciona with the chemical mutagen N-ethyl-N-nitrosourea and Minos transposon [17,32,33,80,81]. Causal genes have been identified in only a few mutants. Most of the mutants generated in the near future would therefore be novel ones. Insertional mutagenesis provides an ideal system in Ciona because the causal genes are identifiable in a short period of time, without time consuming fine mapping.

Conclusion

In this article we review recent achievements in germline transgenesis with the Minos transposon in Ciona intestinalis. These studies have revealed that Minos is a highly active transposon in this organism, as shown by establishment of techniques such as stable transgenic lines, enhancer detection, and insertional mutagenesis. These technical innovations will be of great value to future genetic analyses in C. intestinalis. Frequent enhancer detection by remobilization will provide useful transgenic lines. Insertional mutagenesis allows the identification of novel functions of genes during development, as shown by the example of cellulose synthase. Taking into consideration the advantages of Ciona as a subject of genetics research, future genetic analyses in this organism will provide unique insights into chordate gene function. In addition to these technical innovations with Minos, we describe several technical hurdles that Ciona researchers must overcome if they are to conduct large-scale mutagenesis studies.

Minos is a valuable transposon, and its activity may be the first to be tested in organisms for which no genetic approach has yet been introduced. We also provide evidence that some other TcI/mariner superfamily transposons have excision activity in Ciona. However, these transposons have not been found to be efficient in causing germline transgenesis in Ciona. This information may be useful in elucidating the mechanisms that determine transposon activity in different organisms. Resolving these issues would make these transposons further valuable tools in Ciona genetics research.

Competing interests

The authors declare that they have no competing interests.

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References
1. Rubin GM, Kidwell MG, Bingham PM: The molecular basis of P-M hybrid dysgenesis: the nature of induced mutations. Cell 1982, 29:967-994.
2. Emmons SW, Yesner L, Ruan KS, Katzenberg D: Evidence for a transposon in Caenorhabditis elegans. Cell 1983, 32:55-65.
3. Jacobson JW, Medhora MM, Hardt DL: Molecular structure of a somatically unstable transposable element in Drosophila. Proc Natl Acad Sci USA 1986, 83:6684-6688.
4. Bryan G, Garza D, Hardt D: Insertion and excision of the transposable element mariner in Drosophila. Genetics 1990, 125:110-114.
5. Franz G, Savakis CC: Minos, a new transposable element from Drosophila hydei, is a member of the Tcl-like family of transposons. Nucleic Acids Res 1991, 19:6646.
6. Koga A, Suzuki M, Inagaki H, Bessho Y, Horii H: Transposable element in fish. Nature 1996, 383:30.
7. Frazer MJ, Ciszczon T, Ellick T, Bauser C: Precise excision of TTA-A-specific lepidopteran transposons piggvBac (IFP3) and tagalong (TFP3) from the baculovirus genome in cell lines from two species of Lepidoptera. Insect Mol Biol 1996, 5:141-151.
8. Ivics Z, Hackett PB, Plasterk RH, Izsak Z: Molecular reconstruction of Sleeping Beauty, a Tcl-like transposon from fish, and its transposition in human cells. Cell 1997, 91:501-510.
9. Lampe DJ, Akberly BJ, Rubin EJ, Mekalanos JJ, Robertson HM: Hyperactive transposase mutants of the Himar1 mariner transposon. Proc Natl Acad Sci USA 1999, 96:14428-14433.
10. Kawakami K, Shima A, Kawakami N: Identification of a functional transposase of the Tcl element, an Ac-like element from the Japanese medaka fish, and its transposition in the zebrafish germ lineage. Proc Natl Acad Sci USA 2000, 97:14043-14048.
11. Miskev C, Izsav Z, Plasterk RH, Ivics Z: The Frog Prince: a reconstructed transposon from Rana pipiens with high transpositional activity in vertebrate cells. Nucleic Acids Res 2003, 31:6873-6881.
12. Sasakura Y, Awazu S, Chiba S, Satoh N: Germ-line transgenesis of the Tcl/mariner superfamily transposon Minos in Ciona intestinalis. Proc Natl Acad Sci USA 2003, 100:7726-7730.
13. Sasakura Y: Germline transgenesis and insertional mutagenesis in the ascidian Ciona intestinalis. Dev Dyn 2007, 236:1758-1767.
14. Sasakura Y, Awazu S, Chiba S, Satoh N: Application of Minos, one of the Tcl/mariner superfamily transposable elements, to ascidian embryos as a tool for insertional mutagenesis. Gene 2003, 308:1-20.
15. van Luenen HG, Collins SD, Plasterk RH: The mechanism of transposition of Tcl in C. elegans. Cell 1999, 79:293-301.
16. Awazu S, Sasaki A, Matsuoka T, Satoh N, Sasakura Y: An enhancer trap in the ascidian Ciona intestinalis identifies enhancers of its Musashi orthologous gene. Dev Biol 2004, 275:459-472.
17. Sasakura Y, Nakashima K, Awazu S, Matsuoka T, Nakayama A, Azuma J, Satoh N: Transposon-mediated insertional mutagenesis revealed the functions of animal cellulose synthase in the ascidian Ciona intestinalis. Proc Natl Acad Sci USA 2005, 102:15134-15139.
18. Satoh N: Developmental Biology of Ascidians. New York: Cambridge University Press; 1994.
19. Delsuc F, Brinkmann H, Chourrout D, Philippe H: Tunicates and not cephalochordates are the closest living relatives of vertebrates. Nature 2004, 439:965-968.
20. Bourlat SJ, Julussdottir T, Lowe CJ, Freeman R, Aronowicz J, Kirschcr M, Lander ES, Thordyrkyme M, Nakano H, Kohn AB, et al: Deuterostome phylogeny reveals monophyletic chordates and the new phylum Xenoturbellida. Nature 2006, 444:85-88.
21. Nicol D, Meintzogeh IA: Cell counts and maps in the larval central nervous system of the ascidian Ciona intestinalis (L.). J Comp Neurol 1991, 309:415-429.

22. Nishida H, Satoh N: Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. I. Up to the eight-cell stage. Dev Biol 1983, 99:382-394.

23. Nishida H, Satoh N: Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. II. The 16- and 32-cell stages. Dev Biol 1985, 110:4-9.

24. Nishida H: Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. III. Up to the tissue restricted stage. Dev Biol 1987, 121:526-541.

25. Clooney RA: Ascidian larvae and events of metamorphosis. Ymer Zool 1982; 22:817-826.

26. Ogasawara M, Wada H, Peters H, Satoh N: Developmental expression of Pax1/9 genes in urochordate and hemichordate gills: insight into function and evolution of the pharyngeal epithelium. Development 1999, 126:1539-1550.

27. Ogasawara M, Di Lauro R, Satoh N: Isolation of developmental mutants of the ascidian Ciona savignyi. Mol Gen Res 2005, 15:1668-1674.

28. Satoh N: The ascidian tadpole larva: comparative molecular development and genomics. Nat Rev Genet 2003, 4:285-295.

29. Satoh N, Satou Y, Davidson B, Levine M: Ciona intestinalis: an emerging model for whole-genome analyses. Trends Genet 2003, 19:376-381.

30. Shi W, Lively M, Davidson B: Unraveling regulatory networks in the simple chordate, Ciona intestinalis. Genome Res 2005, 15:1668-1674.

31. Satoh N, Levine M: Surfing with the tunicates into the post-genome era. Genes Dev 2005, 19:2407-2411.

32. Moody R, Davis SW, Cubes F, Smith WC: Isolation of developmental mutants of the ascidian Ciona savignyi. Mol Gen Res 1999, 26:199-206.

33. Nakanati Y, Moody R, Smith WC: Mutations affecting tail and notochord development in the ascidian Ciona savignyi. Dev Genet 1999, 24:292-301.

34. Hendrickson C, Christian L, Desch et K, Jiang D, Joly JS, Legendre L, Nakanati Y, Tresser J, Smith WC: Culture of adult ascidians and ascidian genetics. In vitro 2003, 19:158-169.

35. Nakatani Y, Tresser J, Smith WC: Ascidian genetics. In vitro 2003, 19:158-169.

32. Moore J, Davidson B, Smith WC: Isolation of developmental mutants of the ascidian Ciona savignyi. Mol Gen Res 2005, 15:1668-1674.

37. Loukeris TG, Livadaras I, Arca B, Zabulou S, Savakis C: Genomic insertion of the transposable element Minos into the germ line of Drosophila melanogaster. Proc Natl Acad Sci USA 1995, 92:9485-9489.

38. Klimakis OG, Zagoraiou L, Vassilatis DK, Savakis C: Genome-wide insertion mutagenesis in human cells by the Drosophila mobile element Minos. EMBO Rep 2000, 1:416-421.

39. Klimakis OG, Loukeris TG, Pavlopoulos A, Savakis C: Mobility assays confirm the broad host-range activity of the Minos transposable element and validate new transformation tools. Insect Mol Biol 2000, 9:269-275.

40. Shimizu K, Kamba M, Sonobe H, Kanda T, Klimakis AG, Savakis C, Tamura T: Extrachromosomal transposition of the transposable element Minos in embryos of the silkworm Bombyx mori. Insect Mol Biol 2000, 9:277-281.

41. Zhang H, Shinmyo Y, Hirose A, Inoue Y, Ohuchi H, Loukeris TG, Eggleston P, Noji S: Extrachromosomal transposition of the minos transposable element in zebrafish using the cricket Gryllus bimaculatus. Dev Growth Differ 2002, 44:409-417.

42. Drabek D, Zagoraiou L, deWit T, Lanceveda A, Roumpaki C, Mamalaki C, Savakis C, Grosfeld F: Transposition of the Drosophila hydei Minos transposon in the mouse germ line. Genomics 2003, 81:108-117.

43. Pavlopoulos A, Averof M: Establishing genetic transformation for comparative developmental studies in the crustacean Parathya haewaisani. Proc Natl Acad Sci USA 2005, 102:7888-7893.

44. Metaxakis A, Oehler S, Klimakis A, Savakis C: Minos as a genetic and genomic tool in Drosophila melanogaster. Genetics 2005, 171:571-581.

45. Matsuoka T, Awazu S, Satoh N, Sasakura Y: Minos transposon causes germine transgenesis of the ascidian Ciona savignyi. Dev Growth Differ 2004, 46:249-255.

46. Matsuoka T, Awazu S, Shoguchi E, Satoh N, Sasakura Y: Germine transgenesis of the ascidian Ciona intestinalis by electroporation. Genesis 2005, 41:61-72.

47. Arca B, Zabulou S, Loukeris T, Savakis C: Mobilization of a Minos transposon in Drosophila melanogaster chromosomes and chromatic repair by heteroduplex formation. Genetics 1997, 145:267-279.

48. Davidson AE, Balcunias D, Mohn D, Shaffer J, Hermanson S, Sivabubba S, Clift MP, Hackett PB, Eck SC: Efficient gene delivery and gene expression in zebrafish using the Sleeping Beauty transposon. Dev Biol 2003, 263:191-202.

49. Liu YG, Matsukawa N, Oosumi T, Whittier RF: Efficient isolation and mapping of Arabidopsis thaliana T-DNA insert junctions by thermal asymmetric interlaced PCR. Plant J 1995, 5:457-463.

50. Karsi A, Moav B, Hackett P, Liu Z: Effects of insert size on transposition efficiency of the Sleeping Beauty transposon in mouse cells. Mar Biotechnol 2001, 3:241-245.

51. Fadool JM, Hartl DL, Dowling JE: Transposition of the mariner element from Drosophila mauritiana in zebras. Proc Natl Acad Sci USA 1997, 95:5182-5186.

52. Bessereau JL, Wright A, Williams DC, Schuske K, Davis MW, Jor- gensen EM: Mobilization of a Drosophila transposon in the Caenorhabditis elegans germ line. Nature 2001, 413:70-74.

53. Fischer SE, Wienholds E, Plasterk RH: Regulated transposition of a fish transposon in the mouse germ line. Proc Natl Acad Sci USA 2001, 98:6759-6764.

54. Horie K, Kuroiwa A, Ikawa M, Okabe M, Kondoh G, Matsuda Y, Takeda J: Efficient chromosomal transposition of a Tcl/mariner-like transposon Sleeping Beauty in mice. Proc Natl Acad Sci USA 2001, 98:9191-9196.

55. Zayed H, Izsák Z, Khare D, Heinemann U, Ivics Z: The DNA-binding protein HMGB1 is a cellular cofactor of Sleeping Beauty transposition. Nucleic Acids Res 2003, 31:2313-2322.

56. Walsko O, Izsák Z, Szabo K, Kaufman CD, Harold S, Izsák Z: Sleeping Beauty transposase modulates cell-cycle progression through interaction with Miz1. Proc Natl Acad Sci USA 2006, 103:4062-4067.

57. Cruz NB, Weinreich MD, Wiegand TW, Krebs MP, Reznikoff WS: Characterization of the Ts5 transposase and inhibitor proteins: a model for the inhibition of transposition. J Bact 1993, 175:6932-6938.

58. Corbo JC, Di Gregorio A, Levine M: The ascidian as a model organism, Ciona intestinalis, in developmental and evolutionary biology. Cell 2001, 106:535-538.
69. Keys DN, Lee BI, Di Gregorio A, Harafuji N, Detter JC, Wang M, Kahai O, Ahn S, Zhang C, Doyle SA, et al.: A saturation screen for cis-acting regulatory DNA in the Hox genes of Ciona intestinalis. Proc Natl Acad Sci USA 2005, 102:679-683.

70. Awazu S, Matsuoka T, Satoh N, Inaba K, Sasakura Y: High-throughput enhancer trap by remobilization of transposon Minos in Ciona intestinalis. Genesis 2007, 45:307-317.

71. Balciunas D, Davidson AE, Sivasubbu S, Hermanson SB, Welle Z, Ekker SC: Enhancer trapping in zebrafish using the Sleeping Beauty transposon. BMC Genom 2004, 5:62.

72. Parinov S, Kondrichin I, Korzh V, Emelyanov A: Tol2 transposon-mediated enhancer trap to identify developmentally regulated zebrafish genes in vivo. Dev Dyn 2004, 221:449-459.

73. Dupuy AJ, Fritz S, Largaespada DA: Transposition and gene disruption in the male germine of the mouse. Genesis 2001, 30: 82-88.

74. Shirae-Kurabayashi M, Nishikata T, Takamura K, Tanaka KJ, Nakamoto C, Nakamura A: Dynamic redistribution of vasa homolog and exclusion of somatic cell determinants during germ cell specification in Ciona intestinalis. Development 2006, 133:2683-2693.

75. Robertson HM, Preston CR, Phillips RW, Johnson-Schlitz DM, Benz WK, Engels WR: A stable genomic source of P element transposase in Drosophila melanogaster. Genetics 1988, 118:461-470.

76. Amsterdam A, Burgess S, Golling G, Che W, Sun Z, Townsend K, Farrington S, Haldi M, Hopkins N: A large-scale insertional mutagenesis screen in zebrafish. Genes Dev 1999, 13:2713-2724.

77. Amsterdam A: Insertional mutagenesis in zebrafish. Dev Dyn 2003, 228:523-534.

78. Chen W, Burgess S, Golling G, Amsterdam A, Hopkins N: High-throughput selection of retrovirus producer cell lines leads to markedly improved efficiency of germ line-transmissible insertions in zebra fish. J Virol 2002, 76:2192-2198.

79. Nakashima K, Yamada L, Satou Y, Azuma J, Satoh N: The evolutionary origin of animal cellulose synthase. Dev Genes Evol 2004, 214:81-88.

80. Sordino P, Heisenberg CP, Cirino P, Toccano A, Giuliano P, Marino R, Pinto MR, De Santis R: A mutational approach to the study of development of the protochordate Ciona intestinalis (Tunicata, Chordata). Sarsia 2000, 85:173-176.

81. Sordino P, Belluzzi L, De Santis R, Smith WC: Developmental genetics in primitive chordates. Philos Trans R Soc Lond B Biol Sci 2001, 356:1573-1582.

82. Eukaryotic Genomics [http://genome.jgi-psf.org/]