ORIGINAL ARTICLE

The *Tol1* element of the medaka fish, a member of the *hAT* transposable element family, jumps in *Caenorhabditis elegans*

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Tol1 is a DNA-based transposable element residing in the genome of the medaka fish *Oryzias latipes*, and has been proven to be transposed in various vertebrate species, including mammals. This element belongs to the *hAT* (*hobo/Activator/Tam3*) transposable element family, whose members are distributed in a wide range of organisms. It is thus possible that *Tol1* is mobile in organisms other than vertebrates. We here show that transposition of this element occurs in the nematode *Caenorhabditis elegans*. A donor plasmid containing a *Tol1* element and a helper plasmid carrying the transposase gene were delivered into gonad cells and, after several generations of culturing, were recovered from worms. PCR analysis of the donor plasmid, using primers that encompassed the *Tol1* element, revealed

excision of the *Tol1* portion from the plasmid. Analysis of genomic DNA of the worms by the inverse PCR method provided evidence that *Tol1* had been integrated into the *C. elegans* chromosomes. Vertebrates and *C. elegans* are phylogenetically distantly related organisms in that the former are deuterostomes and the latter a protostome animal. Our results indicate (1) the transposition reaction of the *Tol1* element requires, besides the transposase, no factors from host cells, or (2) the host factors, even if required, are those that are common to protostomes and deuterostomes. The results also have significance for the development of a gene transfer vector and other biotechnology tools for *C. elegans*. *Heredity* (2008) **101**, 222–227; doi:10.1038/hdy.2008.47; published online 28 May 2008

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Introduction

A large number of DNA-based transposable elements have been identified in various organisms, and many of them can be grouped into transposable element families, based mainly on the overall structures and amino-acid sequence similarities of internal genes. Ranges of host organisms differ among families, and dependence of the transposition reaction on host species-encoded factors is considered to be one determinant of the host ranges (cf. Lozovskaya *et al.*, 1995). For example, the *P* element of *Drosophila melanogaster* is prevalent in this species, but absent in its sibling species *D. simulans*. Kimura and Kidwell (1994) initiated experimental populations of these two species containing similar copy numbers of the element, and maintained them under similar environmental conditions, observing a higher copy number in *D. melanogaster* in subsequent generations. As the authors concluded, some host factors would be the most likely explanation for the observed difference.

Of several transposable element families known to date, the *mariner*/Tc1 family is most conspicuous in that member elements are distributed in a wide range of organisms. The *mariner* and Tc1 elements were found in

D. mauritiana (Jacobson *et al.*, 1986) and the nematode *Caenorhabditis elegans* (Emmons *et al.*, 1983), respectively, and related elements are known to occur in diverse taxa, including planarians (Garcia-Fernandez *et al.*, 1995), annelids (Jee *et al.*, 2007), vertebrates (Smit and Riggs, 1996), plants (Jarvik and Lark, 1998) and fungi (Langin *et al.*, 1995). Manifestation of such a wide distribution leads to the inference that elements of this family require no or few factors produced from host cells for their transposition reaction. This has already been demonstrated with *mariner*, Tc1 and some other elements: transposition occurs *in vitro* as long as a purified transposase is provided (Vos *et al.*, 1996; Lampe *et al.*, 1996). It was also inferred and then proven that elements of this family move when they are artificially introduced into organisms other than their original host species. The most notable examples from the viewpoint of the host species distance are their transposition in vertebrates (Figure 1). These elements thus retain the transposition activity even when they cross the protostome/deuterostome boundary. Protostomes and deuterostomes are two main branches of commonly accepted phylogenetic trees for animals (Figure 1). The *piggyBac* element of another family has also been shown to be able to cross this boundary.

There is one other transposable element family whose members are broadly distributed across kingdoms. It is the *hAT* family named for the *hobo*, *Activator* and *Tam3* elements from *D. melanogaster* (McGinnis *et al.*, 1983), the maize *Zea mays* (Fedoroff *et al.*, 1983) and the snapdragon

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Figure 1 Commonly accepted phylogenetic tree for animals. Phyla were arbitrarily selected, and genetic or temporal distances are not reflected in the branch lengths. The items indicated in white letters are transposable elements that have been shown to be active across the protostome/deuterostome boundary. They were tested for transposition activity in organisms of the phyla indicated by the arrows. The recipient species were: *mariner*, zebrafish animals (Fadool et al., 1998); *Tc1*, human culture cells (Schouten et al., 1998); *piggyBac*, mouse animals (Ding et al., 2005); *Tol1*, *C. elegans* animals (present study).

Antirrhinum majus (Coen et al., 1986), respectively. Similar to the case of the *mariner*/*Tc1* family, no or low dependence on host factors is inferred for elements of this family (Calvi et al., 1991), and this has been confirmed at least in plants (Kunze, 1996).

Tol1 is a DNA-based transposable element residing in the genome of the medaka fish *Oryzias latipes* (Koga et al., 1995). We have recently found a medaka fish strain that was highly mutable because of high-frequency transposition of the element, and identified an autonomous *Tol1* copy by analyzing this strain (Koga et al., 2007a). The autonomous copy carried a transposase gene, and a BLAST search of its amino-acid sequence revealed that *Tol1* is an *hAT* element (Koga et al., 2007b). *Tol1* is mobile in many vertebrate species, including the medaka fish (Tsutsumi et al., 2006), zebrafish (manuscript under review), frog (Hikosaka and Koga, 2007), and human and mouse (Koga et al., 2007a). Vertebrates are located on the phylogenetic branch of deuterostomes. The purpose of this study was to examine if *Tol1* can be transposed in a protostome animal. For this purpose, we first generated transgenic *C. elegans* lines bearing a nonautonomous *Tol1* element and the transposase gene. We then analyzed worms for excision of the element from the donor plasmid and reintegration into the *C. elegans* chromosomes. The results clearly indicated the occurrence of both in *C. elegans* cells. Thus, the *Tol1* element is mobile in both protostomes and deuterostomes. Our results thus suggest that no factors are required from host cells, or that the host factors, even if required, are those that are common to protostomes and deuterostomes.

Materials and methods

C. elegans breeding

The Bristol isolate N2 of *C. elegans* was used as a recipient of foreign DNAs. This strain was homozygous for the wild-type *rol-6* allele. Nematode culture at 20 °C and genetic manipulations used standard procedures (Brenner, 1974).

Plasmid construction

We prepared three kinds of plasmids: donor, helper and marker. The *Tol1* element was expected to be excised from the donor plasmid and then integrated into *C. elegans* chromosomes. The helper plasmid had the role of providing the transposase enzyme that catalyzes the excision and insertion reactions. The marker plasmid was used to screen worms for those carrying the donor and helper plasmids after microinjection.

The donor plasmid is a clone of a 4.3-kb-long fragment of the medaka fish genomic DNA, which contains a 1.9-kb-long *Tol1* element and its flanking chromosomal regions (Figure 2). The 1.9-kb element corresponds to the 1.5-kb left- and 0.4-kb right-terminal regions of the 4.4-kb-long, autonomous *Tol1* element, and most parts of the transposase gene are lacking (see Figure 2 of Koga et al., 2007a).

The helper plasmid was generated by cloning the coding region of the *Tol1* transposase cDNA (Koga et al., 2007a) into the *NheI* site of pPD49.78. pPD49.78 contains the promoter region of the *C. elegans* heat-shock protein gene *hsp16-2* (Stringham et al., 1992) and the 3'-terminal region of the *unc-54* gene, the *NheI* site being located between these.

The marker plasmid was pRF4 (Mello and Fire, 1995). This is a clone of the *rol-6* (*su1006*) gene of *C. elegans*, which is dominant over the wild-type allele and causes a twisted body shape to its carrier.

Generation of transgenic lines

The helper plasmid (150 ng μ l⁻¹) and the donor plasmid (15 ng μ l⁻¹) were mixed with the marker plasmid (150 ng μ l⁻¹), and then injected into the gonad of hermaphrodites. DNAs introduced into *C. elegans* by this procedure are known to undergo intermolecular ligation to generate a large extrachromosomal array that contains many copies of the transgenic DNA, which can be partitioned during mitosis (Mello et al., 1991). The array can be expected to contain all three plasmids. Transgenic worms were selected based on the phenotype of the twisted body shape (roller). Two lines, ST1275 and ST1278, which continually segregated progeny exhibiting the roller phenotype, were established. PCR analyses confirmed that both lines contained both helper and donor plasmids (data not shown).

Heat-shock induction of transposase

For each line, two worm cultures were started by placing 10 young adult hermaphrodites exhibiting the roller phenotype on NGM plates seeded with a lawn of OP50 *Escherichia coli*. The two cultures are designated as H (heat-shock treated) and N (nontreated), hereafter. From 12 h after starting the cultures, animals of the H culture were heat-shocked by placing the plate in an air incubator set at 37 °C for 30 min twice a day for 4 days.

Figure 2 Organization of plasmids. Nucleotide blocks included as components of the plasmids are indicated with abbreviations for EMBL sequence files and the nucleotide positions. The abbreviations are: (Tyr) AB010101 (the *tyrosinase* gene of medaka), (Tol1) D42062 (a 1.9-kb-long copy of the *Tol1* element) and (Tpase) AB264112 (cDNA for the transposase). The black triangles at the ends of the *Tol1* element indicate terminal inverted repeats of 14 bp. The open triangles show locations and directions of the PCR primers. P1s are for detection of excision, and P2s for insertion.

During the heat-shock treatments, animals of the N culture were left in a 20 °C incubator. The N culture had the role of a negative control for the transposase function.

At 12 h after the final heat-shock treatment, animals were collected by washing the plates with distilled water.

Analysis of donor plasmid for excision of *Tol1*

DNA was extracted from the worms with the Puregene DNA purification kit (Gentra Systems Inc., Minneapolis, MN, USA). Using these DNA samples as a template, we conducted a PCR analysis of the donor plasmid using the ExTaq polymerase (Takara Bio Inc., Otsu, Japan) for excision of the *Tol1* element. The primers used were P1L

(nt 3594–3623 of EMBL file AB010101) and P1R (nt 3895–3866). The distance between these two primers on the donor plasmid was 2.2 kb. Precise excision of the *Tol1* portion, which is 1.9 kb in length, from the donor plasmid was expected to result in a PCR product of 0.3 kb. The PCR conditions were as follows: (94 °C, 120 s), 46 × (94 °C, 20 s; 64 °C, 20 s; 72 °C, 15 s), (72 °C, 60 s).

Analysis of genomic DNA for insertion of *Tol1*

We used the inverse PCR technique to detect insertion. As the first step, we digested 4.0 µg of DNA with *Bam*HI, *Eco*RI or *Xba*I and *Nhe*I. Because the *Tol1* portion of the donor plasmid did not contain cutting sites for these restriction enzymes, the digestion treatment was expected to yield restriction fragments consisting of the entire *Tol1* element and its flanking chromosomal regions. We electrophoresed the digested DNAs on a 1.0% agarose gel, cut out a gel piece containing 1.9–6.0 kb DNA fragments and recovered the fragments from the gel piece. This was to exclude fragments of <1.9 kb that were too small to contain the entire *Tol1* element and those of >6.0 kb that included fragments too long to be amplified by inverse PCR.

Size-selected DNA fragments were ligated with T4 DNA ligase, concentrated through ethanol precipitation, and then used as a template for inverse PCR. The primers for this PCR analysis were P2L (nt 1758–1787 of EMBL file D42062) and P2R (nt 130–101). The PCR conditions were as follows: (94 °C, 120 s), 40 × (94 °C, 20 s; 64 °C, 20 s; 72 °C, 150 s), (72 °C, 180 s). The obtained PCR products were cloned into pT7Blue-2 (Takara Bio Inc.) and sequenced.

Insertion sites of *Tol1* elements in the *C. elegans* genome were determined by using *C. elegans* Blast Server (http://www.sanger.ac.uk/cgi-bin/blast/submitblast/c_elegans).

Results

Detection of excision by PCR

We conducted a PCR analysis of DNAs extracted from worms bearing transgenes containing the donor plasmid, and the reaction mixtures were electrophoresed on an agarose gel. With both lines, the DNA sample from the H culture produced DNA fragments of about 0.3 kb, and no PCR product was observed in the lane for the N culture (data not shown). It was thus suggested that excision of the *Tol1* element occurred in worms of the H cultures. The results do not necessarily mean that excision did not occur in worms of the N cultures, but the frequency was, even if excision occurred, lower than in worms of the H cultures.

Confirmation of excision by sequencing

To determine the breakpoints of the rearrangements of the donor plasmid, we cloned PCR products from the ST1275-H and ST1278-H cultures. Several clones were available from each line, but only one clone was chosen at random. The two clones were then sequenced along both strands (Figure 3). In both clones, the entire *Tol1* sequence was found to have been removed and parts of the target site duplication (TSD) sequences had been left behind. In addition, 8-bp- and 15-bp-long sequences had been inserted at the breakpoints. These extra nucleotides

Figure 3 Nucleotide sequences of excision breakpoints and their vicinities. The top line is the sequence of the donor plasmid, shown for reference. The other two lines are the sequences of cloned PCR products. Dots indicate there are nucleotides not shown here.

appear to be unrelated to the *Tol1* sequence, and consist of sequences complementary to the TSD sequence.

The deletion of the entire *Tol1* region provides clear evidence for the occurrence of excision. The excision was not strictly precise in both samples, as has commonly been observed with *Tol1* (Koga *et al.*, 2007b; Hikosaka and Koga, 2007).

Detection of insertion by inverse PCR

DNAs extracted from worms of the H cultures were digested with restriction enzymes, ligated with T4 DNA ligase and then used as templates for inverse PCR analysis. Several PCR products of different sizes were observed in single lanes, suggesting that the DNA samples contained more than one insertion at different chromosomal locations (data not shown). Worms of the N cultures were not included in this analysis because our purpose was to demonstrate the occurrence of insertion and this was likely to be attained with only worms of the H cultures.

Confirmation of insertion by sequencing

The PCR products of each lane were cloned into plasmids and sequenced. Of four clones examined, one appeared to originate from the donor plasmid integrated into the extrachromosomal array because the sequences of the *Tol1*-flanking regions were identical to those of the donor plasmid. The other three clones were found to have *Tol1*-flanking regions whose sequences were different from those of the donor plasmid (Figure 4). A BLAST search against the *C. elegans* nucleotide sequence database revealed that the left- and right-flanking regions were adjacently located in single sequence files. The sequences of clones a, b and c were found to be included in the sequences of the fosmid clone designated as H10E21 (tip of chromosome 3, nucleotide identity 100%), cosmid clone T19B10 (one-third of the chromosome length from one end of chromosome 4, identity 98%) and cosmid clone F19B6 (middle of chromosome 5, identity 99%), respectively. No coding sequence was predicted at their insertion sites. In clones a and b, 8-bp sequences on the left side and the right side of the *Tol1* element were identical, which indicated that TSD characteristic of insertion of the *Tol1* transposition in the medaka fish genome also took place in *C. elegans*. Clone c did not have an apparent TSD.

The above findings that the *Tol1* element was inserted in three different *C. elegans* genomic sequences indicate that the *Tol1* element had been inserted into the chromosomes by transposition.

Discussion

Transposition of *Tol1* in a protostome

In the evolutionary stage of acquisition of triploblastic embryogenesis by diploblastic metazoans, they diverged into the two branches of protostomes and deuterostomes. In the present study, we have demonstrated that the *Tol1* element native to a deuterostome animal (medaka fish) is transposed in cells of a protostome animal (*C. elegans*). Our results indicate that host-cell-encoded factors indispensable for the transposition reaction of the *Tol1* element are, even if required, those that are common to protostomes and deuterostomes. This could be helpful in explaining why *hAT* elements are distributed in a wide range of organisms, including both protostome and deuterostome animals. We have previously shown evidence for horizontal (interspecific) transfer of *Tol2*, another *hAT* element of the medaka fish, between two species of the genus *Oryzias* (Koga *et al.*, 2000). The present results support the view that horizontal transfer of *hAT* elements might occur between more distantly related animals.

Transmission of insertions across generations

The results of the inverse PCR analysis suggest that *Tol1* elements inserted in the *C. elegans* chromosomes were transmitted across generations.

Electrophoresis of the inverse PCR products exhibited distinct bands in the present study. We have previously applied exactly the same procedures to human culture cells (Koga *et al.*, 2007b). On conducting inverse PCR with cells after proliferation through several rounds of cell division, we observed smeared PCR products. This result indicates that there were a great number of insertions of different origins, each being low in frequency. We then prepared cell clones from single cells. Inverse PCR with these cells, in contrast, resulted in a few distinct bands in electrophoresis photos. These results suggest that in the present study the *Tol1* insertions passed through a bottleneck after their occurrence. Such a bottleneck is likely to have arisen through changes in generations. A possible scenario is as follows: insertions that occurred in germ-line cells and/or undifferentiated blastomeres were transmitted to subsequent generations through germ cells, and amplified in somatic cells of the animals used for DNA preparation.

Possibility of transposition in other taxa

The findings that the *Tol1* element is transposed in chordate and nematode phyla suggest the mobility of this element in other animals located at phylogenetic

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Donor   GGGGCCCTGTCTGGTCGCGGGTTCTGTGCAGACGTCTCGGTTTCAGAC
Clone a CATAAAACCTGGTGCTAAACACGATAAACTTATTGAAACATGGCACAC
Clone b TGATACAGAATTAGCAGAACTACAGGGGCCCTGTTTCTCTCGGGTCT
Clone c AATAGAGTTAAAGATTTTGATTCAAACCTCCATAGTAGACAGGCATA

GAGCCCAACGGGCGCAGTACCCTCACAGCGGGATCGATGACAGGGAG
ACCTGAAATTTTCCGATTTTGAAAAAGTGCTCAGCAGGTACCCCGAGC
TTGCACACTTTTTTCAAAAAAGGGGTCTTAGGGTCTCGCAATTCTAT
TCTTTATGCCTACCTAGGCGCAGTCTACTTTTTGGTTTTGGTGTGGTG

CGCTGGCCTTTAGCCAGTAGCGGTTCTAGGCACGGGCCGTCCGG...
TGGACCACTGGGGTCAGTAGCGGTTCTAGGCACGGGCCGTCCGG...
CTAGATCTGGGAAACAGTAGCGGTTCTAGGCACGGGCCGTCCGG...
TTAGGTTGATCTTTCCGTAGCGGTTCTAGGCACGGGCCGTCCGG...

...GGGGAGTAATTCAGGGTAGAACCGCCACTGCCTTTAGCTTTCTT
...GGGGAGTAATTCAGGGTAGAACCGCCACTGACTGGGGTGTCCAG
...GGGGAGTAATTCAGGGTAGAACCGCCCTGCTGGGAAATTTTCAG
...GGGGAGTAATTCAGGGTAGAACCGCCACTGATCACCACCCAGTA

CAACCGGACGTGTCGTTGTGCAGGAACTATGGAGGGTTTAACTGTGG
AAGGGTACCCCGTTATATTGAAAAATTTGGGCCCTCTGTCATGTTTAA
TAATACCTGAAAACCAATAACCTAGGAATATTGGGAGACGTTGTCCA
TAAAACCACTGAAAATTACGTACCAACAACGTTATTTTTCTAAACAA

GGAATGCAGATTCGGTTACTGGGGCTCCAACTGTGCTGAGTACAGAGA
CCAATTTTTTTGAATTCCAAGACCCTAAATTTTTTCCAATTTTTTACC
AGAAGAAAAGTTTTTGGCATTTCAGAAATAGGTAAAATATTTCCAAAT
AAACAACAACGTTATTTTTCTAAATAACTGCATGAACAATAAATGAAT

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Figure 4 Nucleotide sequences around insertion sites. The top line is the sequence of the donor plasmid, shown for reference. The other three lines are sequences of clones of three different inverse PCR products. Clones a and b originate from PCR reactions with ST1275/*Eco*RI, and clone c from ST1275/*Bam*HI. The black and gray shaded regions are the *Tol1* terminal regions and the target site duplications (TSDs), respectively. Clone c does not have an apparent TSD.

positions closer to the origin of animals, including echinoderms, flatworms and even diploblastic animals, such as cnidarians. Moreover, it is inferable that the element is mobile in other higher organisms, such as arthropods and molluscs (Figure 1). If this is proven, we can hold the view that the independence of *hAT* family elements from host-species-specific factor(s) is as low as that of *mariner*/*Tc1* family elements.

Significance in biotechnology applications

Transposable elements are used as powerful tools for basic and translational research. Applications include gene transfer, mutagenesis and gene/promoter/enhancer trapping. In *C. elegans*, the *Tc1* element, which was identified originally in this species, is transposed efficiently, and has been utilized for insertion mutagenesis (Plasterk and van Luenen, 1997; Bessereau, 2006). Using native *Tc* transposons, including the *Tc1* element, as mutagens, however, has some drawbacks: mobilization of *Tc* transposons is not restricted to a single class of elements. In addition, the *C. elegans* genome harbors plenty of its copies that complicate the identification of the mutagenic insertion. Using exogenous transposon systems would be a way to circumvent these limitations. Indeed, the *mariner* element isolated from *D. mauritiana*

has been demonstrated to be mobile in *C. elegans* (Bessereau *et al.*, 2001), and has served as a useful tool for tagged mutagenesis.

The *Tol1* element has already been developed as a gene transfer vector for vertebrates (Koga *et al.*, 2007a). Our present results on its mobility in *C. elegans* have paved the way for the development of a superior gene transfer vector and other genetic tools for this useful model organism. Considering the possible mobility of this element in a wide range of animals, technologies already developed for vertebrates and those to be developed for *C. elegans* might be directly applicable to various animals.

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