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Characterization of a transcriptionally active Tc1-like transposon in the microsporidian *Nosema bombycis*

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Abstract

The Tc1 transposable element has been found in a wide variety of organisms including vertebrates, insects and fungi but has not been previously reported in Microsporidia. In this study we characterize an intact DNA transposon (*NbTc1*) from the microsporidian *Nosema bombycis*. This transposable element encodes a 337 amino acid transposase sequence, which contains the D,D34E functional motif required for transposition. A Southern blot of *N. bombycis* DNA separated by pulsedesis shows that copies of the *NbTc1* transposon are present on 10 of the 14 chromosomes of *N. bombycis*. Amino acid sequence variation among copies of the *NbTc1* is low, suggesting a conserved function for this transposon within *N. bombycis*. Phylogenetic analysis indicates that *NbTc1* is a new member of the Tc1 family lineage, quite distinct from all previously described Tc1 elements, including those from fungi, indicating that *NbTc1* forms a unique clade of the Tc1 superfamily. However, the Tc1 transposon is too divergent to resolve the major phylogenetic relationships among these superfamilies. Reverse transcriptase PCR and Solexa sequencing suggest that *NbTc1* possesses transcriptional activity. Considering the interest in Microsporidia as biological control agents, the *NbTc1* transposon may be a useful vector for the efficient transfection of these important parasites into host species.

Keywords

Nosema bombycis, Tc1-like, transposon, transposase, genome

Introduction

Transposable elements are an important part of most eukaryotic genomes, and are grouped into two classes based on their mechanism of movement within a genome. Class I elements are retrotransposons, replicating via an RNA intermediate, while class II elements are DNA transposons, moving via a DNA-mediated cut-and-paste mechanism. Tc1/mariner elements are class II transposons found in diverse taxa including fungi, flies, nematodes, fishes, and mammals (Plasterk and Luenen 2002). These elements usually encode a transposase with a DDE/D catalytic motif (i.e., two aspartic acid residues followed by a glutamic acid or an aspartic acid residue, all in conserved positions) which is flanked by short terminal inverted repeats (TIRs) and by target site duplication (TSD) of the dinucleotide TA. Tc1/mariner elements can be classified into several monophyletic clades based on variations of the catalytic motif which include D,D34D, D,D34E, D,D37D, D,D37E, and D,D39E (Shao and Tu 2001, Robertson 2002, Silva *et al*. 2005), suggesting that all Tc1/mariner elements were derived from a common ancestor and may transpose through similar mechanisms. The first Tc1 element to be identified was isolated from *Caenorhabditis elegans*. This 1,610 bp transposon, which contains two 54 bp TIRs, was identified as a repeated sequence responsible for some of the polymorphisms seen among different strains of *C. elegans* (Emmons *et al*. 1983, Liao *et al*. 1983, Rosenzweig *et al*. 1983). Further studies have shown that Tc1 mobilization can be utilized to inactivate genes by insertional mutagenesis and to introduce foreign genes into a species (Zwaal *et al*. 1993, Schouten *et al*. 1998). Many higher animals including vertebrates and insects also possess multiple families of Tc1-like DNA transposons (Avancini *et al*. 1996). Sleeping Beauty is a wide host-range transposon derived from salmonids, which has been used as a vector for genetic transformation in vertebrates (Ivics *et al*. 1997, Izsvak *et al*. 2000, Starr and Largaespada 2005). Minos, a transposon from *Drosophila hydei*, has been shown to be mobile in other fruit fly species and in mammalian tissues (Franz and Savakis 1991, Zagoraiou *et al*. 2001). Although Tc1 elements are widespread in animal genomes, most elements iso-

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lated to date are not active, with the exception of the elements isolated from *Caenorhabditis* and *Drosophila* species, which are apparently intact and without frameshift mutations, insertions, deletions or premature termination codons in the transposase intragenic region.

Microsporidia are obligate intracellular parasites of higher eukaryotes which infect a wide variety of vertebrate and invertebrate hosts. More than 1,200 species of Microsporidia, belonging to 150 genera, have been reported (Wittner 1999). Evidence from a variety of molecular phylogenetic studies supports a fungal origin for the Microsporidia (Hirt *et al*. 1999, Keeling *et al*. 2000, Thomarat *et al*. 2004). Some studies indicate that Microsporidia may be derived from an endoparasitic chytrid ancestor similar to *Rozella allomycis* (James *et al*. 2006). Other studies suggest a zygomycete origin for the Microsporidia (Keeling 2003, Lee *et al*. 2008).

Sequence segments coding for fragments of reverse transcriptase or complete retrotransposons have been reported in the microsporidian species *Spraguea lophii*, *Vittaforma corneae*, *Edhazardia aedis* and *Brachiola algerae* (Hinkle *et al*. 1997, Mittleider *et al*. 2002, Williams *et al*. 2008). Eight complete long terminal repeat (LTR) retrotransposons have been identified in the genome of *Nosema bombycis* (Xu *et al*. 2006). In an express sequence tag (EST) survey of the microsporidian *Edhazardia aedis*, Gill *et al*. (2008) documented the transcription of a Ty3/Gypsy retrotransposon. This was the first documented example of a transposable element transcript in the Microsporidia. Although an increasing number of retrotransposons have been reported in the Microsporidia, only one example of a DNA transposon in the Microsporidia has previously been reported (Williams *et al*. 2008). Several short fragments in a genome sequence survey of *Brachiola algerae* were identified as putative transposase sequences (Williams *et al*. 2008), but no complete transposon sequences were found. In this study, we describe *NbTc1*, a complete DNA transposon found in *Nosema bombycis*, which contains the D,D34D catalytic motif and copies of which are found on ten of the fourteen chromosomes visualized by PFGE in the genome of *N. bombycis*. Sequence and phylogenetic analyses demonstrate that *NbTc1* is a distinct representative in the Tc1 family lineage. Both the low degree of structural variation among *NbTc1* copies and the presence of a *NbTc1* transcript suggest that this element may be functional. We believe that this to be the first transcribed Tc1/mariner type transposable element to be described in a Microsporidia, and suggest that it may have valuable applications as a transformation vector for the Microsporidia.

Materials and methods

Spore production and purification

Nosema bombycis isolate CQ1, obtained from infected *Bombyx mori* silkworms in Chongqing, China, and was preserved at the China Veterinary Culture Collection Center (CVCC, No. 102059). Third instar silkworm larvae were fed mulberry leaves which had been artificially contaminated with *N. bombycis* spores (about 2.0×10^5 spores per silkworm). The silk glands of the infected larvae were dissected on day 4 or 5 of the fifth instar, and were then homogenized and centrifuged to pellet the spores. Spores were resuspended and purified in a discontinuous Percoll gradient (25%, 50%, 75%, and 100%, v/v) and centrifuged at 30,000 *g* for 40 min. The resulting pellets of mature spores were rinsed and stored at 4°C.

Preparation of probes

Nosema bombycis genomic DNA (3 µg) was extracted using the CTAB method (Li and Wang 2006). Two primers (*N. bombycis NbTc1*, 5'-GTGGGGTTGTTTCTCATACTA-3' and 5'-ACCTCCTTTTGCTCGATGTAC-3') were designed and synthesized (Sangon Co, Shanghai, China) to amplify a 425 bp fragment between positions 842 and 1265 of *NbTc1*, which contains the catalytic motif of the transposase. PCR amplifications were performed using an Eppendorf Mastercycle apparatus. Each 50 µl reaction contained 1 µl genomic DNA (50 ng/µl), 2 µl of each primer (10 µmol/L), 2 µl dNTPs (10 mmol/L), 2 µl $MgCl_2$ (25 mmol/L), 5 µl 10 × PCR buffer and 1 µl Taq DNA polymerase (5 U/µl, TaKaRa Co, Japan). Samples were heated to 94°C for 5 min to denature the DNA, then 30 cycles were run with 1 min of denaturation at 94°C, 30 s of annealing at 57°C and 2 min of extension at 72°C. The final extension step was carried out at 72°C for 10 min. The PCR products were purified using a DNA Fragment Quick Purification/Recover Kit (DingGuo, China). The recovered DNA fragments were quantified spectrophotometrically (GeneQuant Pro, Biochrom Ltd, UK) and then were used to prepare DIG-labeled random primed probes (DIG DNA Labeling and Detection Kit, Roche, Switzerland).

Pulsed field gel electrophoresis and Southern blotting

Pulsed field gel electrophoresis (PFGE) was performed as follows: about 1×10^9 purified spores were embedded in 1.4% low melting point agarose per plug. The plugs were then incubated first in solution I (0.1 M EDTA, 5% SDS, 0.1 M DTT) for 2 h, then in solution II (0.5 M EDTA, 0.5% SLS, 1.5 mg/mL proteinase K) for 48 h at 50°C. PFGE gels were run on a Gene NavigatorTM System (Amersham Pharmacia Biotech, Piscataway, NJ, USA) in 0.5 XTBE buffer (45 mM Tris base, 45 mM boric acid, 1 mM EDTA) at 5 V/cm, 12°C, with the following pulse times: 60 s for 9 h, 65 s for 12 h, 70 s for 5 h, 75 s for 13 h , 80 s for 7 h , 90 s for 14 h, with a total run time of 60 h. Chromosomal DNA bands were pretreated with UV and then transferred to a nylon membrane using a VacuGeneXL blotting apparatus (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Southern blotting was carried out according to the manufacturer's instructions (DIG DNA Labeling and Detection Kit, Roche) using the DIG-labeled *NbTc1* probes prepared as described above.

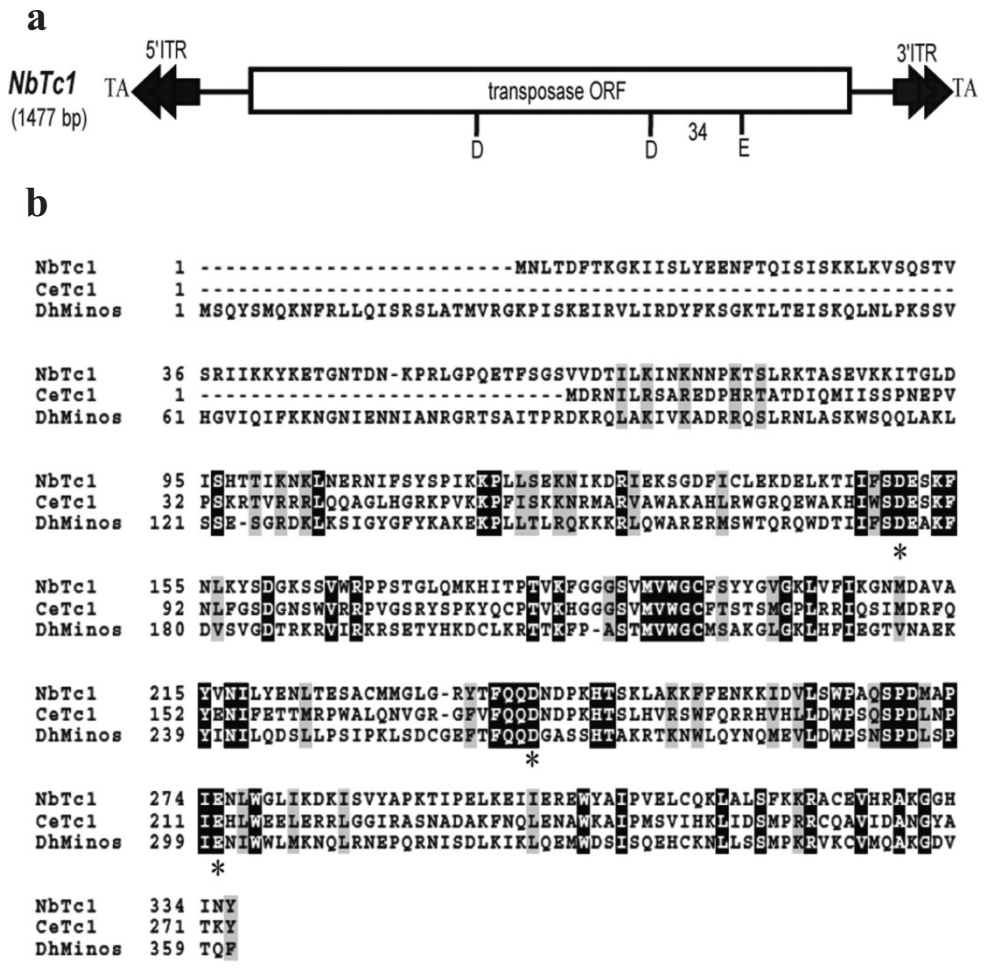

Fig. 1. The structure and sequence of the *Nosema bombycis* Tc1-like element, *NbTc1*: (**a**) Diagram of the *NbTc1* element: ITRs (double arrows), ORF (open box) and the amino acid residues (D,D34E) that represent the catalytic motif; (**b**) The *NbTc1* ORF was aligned with the ORFs of two active Tc1-type elements, Tc1 from *Caenorhabditis elegans* and Minos from *Drosophila melanogaster*. Multiple alignments for three sequences were generated by clustal X (Thompson *et al*. 1997), and positions at which all three amino acid residues are identical are reverse highlighted. Positions at which two of the three amino acid residues are the same are lightly shaded. Each residue in the D,D34E catalytic motif is indicated by an asterisk (∗) below the residue

RT-PCR

Total RNA was isolated from both non-infected (after 72 h in culture) and infected (72 h after infection with *N. bombycis* spores) BmE-SWU1 host cells. Total RNA was extracted with Trizol reagent and treated with DNase I (Invitrogen, California, USA), and mRNAs were reverse transcribed using an oligo(dT)$_{12\text{-}18}$ primer and 15 U of AMV reverse transcriptase (TaKaRa, Dalian, Japan). PCR reactions were run using a 1:2 dilution of the cDNA product of the reverse transcription reaction, which was denatured at 94°C for 5 min, followed by 30 cycles of PCR: 94°C for 1 min, 57°C for 30 s and 72°C for 2 min, followed by a 10 min final extension step at 72°C.

Primers used for the PCR reactions were the *NbTc1* nucleotide primers described above (*N. bombycis NbTc1*, 5'-GTGGGGTTGTTTCTCATACTA-3' and 5'-ACCTCCTTTTGCTCGATGTAC-3'; 425 bp amplicon), and as a positive control, the *N. bombycis* β tubulin primers 5'-TGGACGCCATTAGACAAG-3' and 5'-TCTAAAGACAGCAGCCAC-3' (737 bp amplicon).

A database of expressed sequence tags was constructed using a Gene Expression Sample Prep Kit (Illumina, San Diego, USA). Approximately 2 µg total RNA was extracted from extruded *N. bombycis* spores with Trizol reagent and treated with DNase I. mRNAs were isolated by binding total RNA to magnetic oligo(dT) beads. Using the mRNA attached to the magnetic beads as a template, oligo(dT)-linked cDNAs were synthesized, forming a bead-bound mRNA/cDNA hybrid. The second cDNA strands were then synthesized, and the resulting cDNAs were digested with *NlaIII* and linked to GEX *NlaIII* Adapter 1. The cDNAs were then further digested with *MmeI* and linked to GEX *MmeI* Adapter 2. Finally, the adapter-linked cDNA constructs were enriched by PCR and sequenced using Solexa sequencing technology (BGI, Shenzhen, China).

Sequence analysis and phylogenetic analyses

The *NbTc1* transposon sequence was obtained from the preliminary *N. bombycis* genomic dataset initiated in our labs (Xu *et al*. 2006). The intact *NbTc1* element was detected by performing tBlastn searches of the genome using the Tc1/mariner transposase sequence of other species, with a cut-off of 40% similarity at the amino acid level. Then nucleotide sequences with homology to the transposase were aligned to include the inverted terminal repeats (ITRs) at both ends. The characteristic target site duplications (TSDs) were identified visually. The local Blastn program was run against our genomic database to identify all complete and partial copies of *NbTc1* by retrieving all high-scoring pairs (HSPs) longer than 150 nucleotides and with identities higher than 80%. The copies of *NbTc1* were aligned using Clustal X and manually checked using the data editor MEGA4.0 (Tamura *et al*. 2007). The degree of variation among *NbTc1* copies was estimated from this alignment as the average number of pairwise differences between copies using MEGA4.0.

The *NbTc1* amino acid sequence was aligned with 23 previously reported transposase sequences using Clustal X (Thompson *et al*. 1997). Bootstrap analysis was accomplished with 1,000 replicates using the Maximum Likelihood option of the tree-puzzle software (Schmidt *et al*. 2002). The WAG + G + F model of amino acid substitution was chosen as the most appropriate model with the MODELGENERATOR Program (Keane *et al*. 2006). The *NbTc1* element and its each copy from *N. bombycis* described here has been submitted to Genbank, accession numbers FJ824609 and GQ411164-GQ411188.

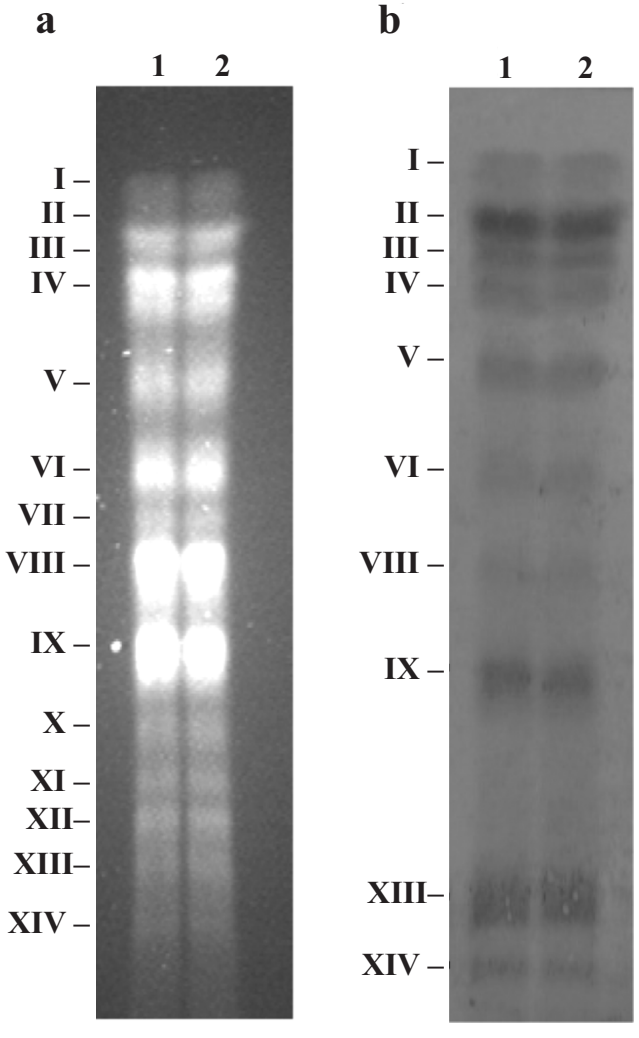

Fig. 2. Distribution of *NbTc1* elements in the *Nosema bombycis* genome: (**a**) *N. bombycis* chromosomal DNA separated by PFGE. Fourteen chromosomal bands are visible (I–XIV). Lanes 1 and 2 are duplicate samples. (**b**) Southern blot analysis of *N. bombycis* chromosomal DNA separated by PFGE. Genomic DNA was hybridized with a 425 bp DIG-labeled *NbTc1* DNA probe. Chromosomal bands that hybridized to the *NbTc1* probe are labeled with Roman numerals. Lanes 1 and 2 represent duplicate samples

Results

Isolation and characterization of NbTc1 in N. bombycis

An initial screening of the *N. bombycis* genome identified several contigs with sequence similarity to Tc1-like trans-

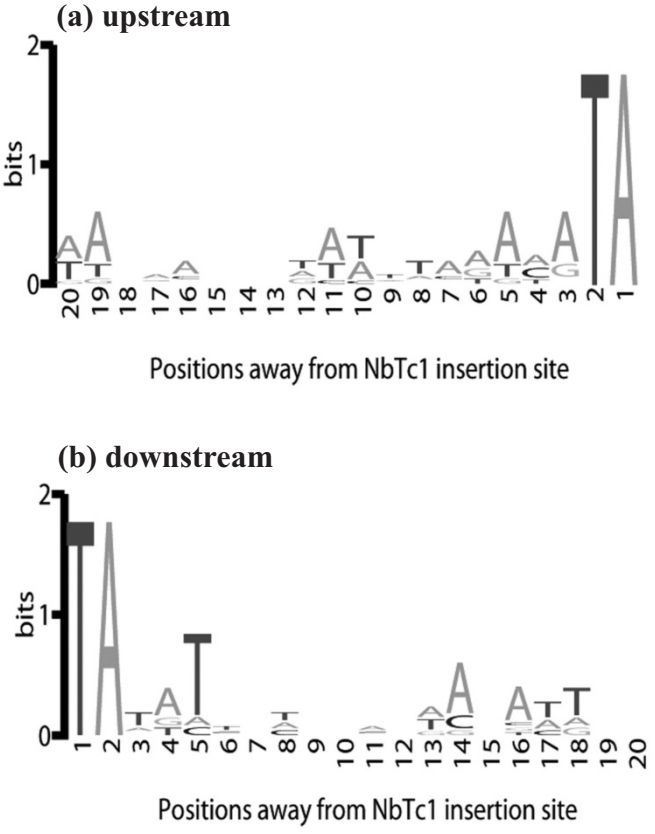

Fig. 3. Sequence logo of the regions flanking complete *NbTc1* insertions. The x-axis represents 20nt upstream (**a**) or downstream (**b**) of the *NbTc1* insertion site; the y-axis is proportional to the level of sequence conservation at each position, with a maximum value of 2

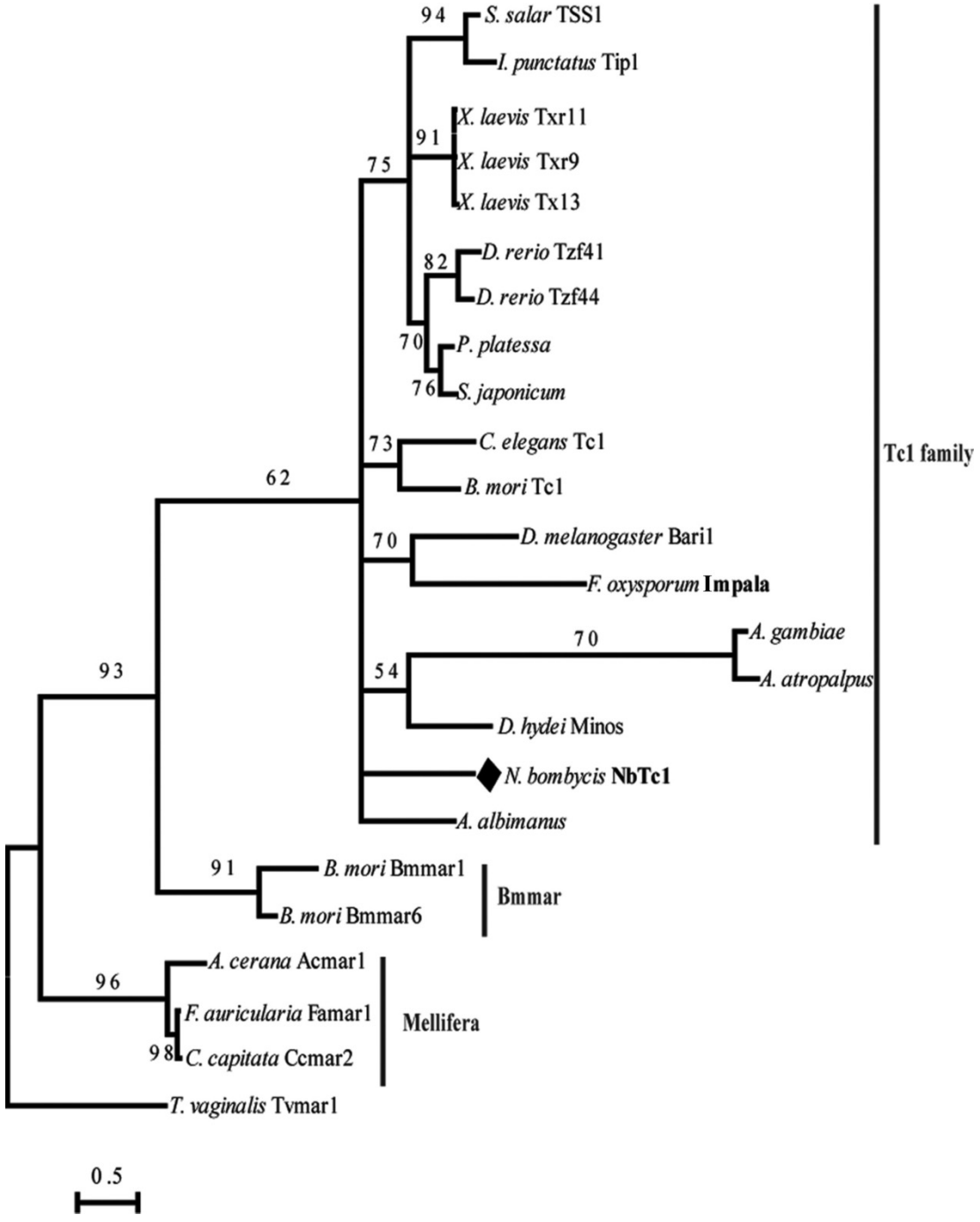

Fig. 4. Phylogenetic position of *NbTc1* and homologues in the Tc1/mariner superfamily. Elements are identified by host name. Percent bootstrap support for Tc1 families and mariner subfamilies are presented. GenBank accession numbers of these elements are as follows: *Xenopus laevis* Txr11 (U43663), *Xenopus laevis* TXr9 (U43662), *Anopheles albimanus* (L76231), *Caenorhabditis elegans* Tc1 (X01005), *Drosophila hydei* Minos (Z29102), *Drosophila melanogaster* Bari1 (X67681), *Salmo salar* TSS1 (L12206), *Ictalurus punctatus* Tip1 (DQ318916), *Pleuronectes platessa* (CAB51372), *Danio rerio* Tzf44 (U51229), *Danio rerio* Tzf41 (U51228), *Bombyx mori* Tc1 (AADK01002315), *Anopheles gambiae* (AF378002), *Aedes atropalpus* (AF377999), *Bombyx mori* Bmmar1 (BMU47917), *Bombyx mori* Bmmar6 (AF461149), *Forficula auricularia* Famar1 (AY155492), *Ceratitis capitata* Ccmar2 (AY155493), *Trichomonas vaginalis* Tvmar1 (AY282463), *Apis cerana* Acmar1 (AB081476), *Fusarium oxysporum* Impala (AF282722), *Schistosoma japonicum* (AAX25877)

posase genes from several eukaryotic species. A complete Tc1 element, *NbTc1*, was identified by searching transposase-containing contigs for the presence of ITRs and target-site duplication (TSDs) that flanks the transposase region. The 1479 bp *NbTc1* element identified here contains 26 bp ITRs flanking, a single open reading frame (ORF), and the TA dinucleotide TSD typical for Tc1 elements (Fig. 1). The putative ORF encodes 337 amino acid residues and possesses the D,D34E cat-

Table I. Location within the *NbTc1* element of two *NbTc1*-specific expressed sequence tags

No. of cDNA	Length of tag (bp)	Matching region of *NbTc1*	Identities
1	44	309–352	97%
2	44	772–814	97%

alytic motif. Multiple alignments show similarity between *NbTc1* and two active transposable elements, Tc1 from *C. elegans* and Minos from *D. melanogaster*.

Genomic distribution of NbTc1 in N. bombycis

To determine the chromosomal distribution of *NbTc1* elements in the *N. bombycis* genome, *N. bombycis* chromosomes were separated by PFGE, and Southern blot analysis was performed, using a 425 bp *NbTc1* fragment (positions 824 to 1265) as a probe. Ten of the fourteen chromosomal bands visualized by PFGE hybridized to the *NbTc1* probe (Fig. 2). These results indicate that *NbTc1* elements are widely distributed among the chromosomes of *N. bombycis* and suggest that transposition of *NbTc1* has occurred between chromosomes.

To further assess the distribution of *NbTc1* in the *N. bombycis* genome, the complete sequence of this element was compared to all *N. bombycis* genomic contigs using BLAST, resulting in the identification of 26 *NbTc1* copies. 50% (13/26) of the *NbTc1* copies were intact (intact defined here as containing at least 95% of the complete *NbTc1* sequence), and 70% of the copies (18/26) included the region containing the D,D34E catalytic motif. The average pairwise difference among Tc1 copies was calculated to be 0.056, suggesting a high level of structural conservation among the *NbTc1* copies.

The flanking regions upstream and downstream from each complete *NbTc1* element were analyzed. All sequences adjacent to the 5' or 3' ITR contained a TA dinucleotide, the typical target site for Tc1/mariner elements which is duplicated when insertion of the transposon occurs. The three nucleotides adjacent to the TA dinucleotide target site showed some sequence conservation, with TAT (upstream) and AA/CA (downstream) being the most common sequences (Fig. 3).

Phylogenetic analysis of N. bombycis *NbTc1*

It is clear from Figure 4 that *NbTc1* belongs to the Tc1 family lineage but is distantly related to the Tc1 element from the other 24 species presented here. Two Tc1/mariner elements (*Impala* and *Fot1*) have been reported from the fungus *Fusarium oxysporum* (Daboussi *et al*. 1992, Langin *et al*. 1995). However, our analysis indicates that the *Impala* element is not closely related to *NbTc1*, and the *Fo1* transposase (Genbank accession no. X64799) shows no homology with *NbTc1* at the amino acid level. At the same time, Figure 4 fails to resolve any of the major lineages indicating that the Tc1 transposase is not a good molecular tool for examining differences among distantly related taxa.

Transcriptional activity of NbTc1

To determine whether the *NbTc1* element is transcribed in *N. bombycis*, polyA RNA was extracted from *N. bombycis* spores, and RT-PCR was run using primers specific for the

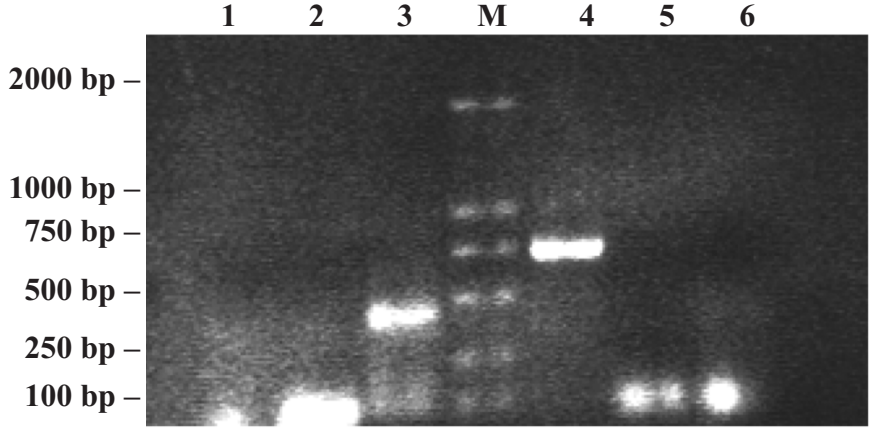

Fig. 5. *NbTc1* transcription in BmE-SWU1 host cells infected with *N. bombycis*. RT-PCR was performed using mRNA isolated from host cells 72 h after infection with *N. bombycis*, and primers specific for *N. bombycis NbTc1* or beta-tubulin. **M**: DL2000 markers (TaKaRa Biotechnology Co); lane **1**: Control – RT-PCR of mRNA from infected host cells with *NbTc1* primers but no reverse transcriptase; lane **2**: Control – RT-PCR of mRNA from non-infected cells with *NbTc1* primers; lane **3**: RT-PCR of mRNA from infected host cells with *NbTc1* primers; lane **4**: RT-PCR of mRNA from infected host cells with beta-tubulin primers; lane **5**: Control – RT-PCR of mRNA from non-infected cells with beta-tubulin primers; lane **6**: Control – RT-PCR of mRNA from infected host cells with beta-tubulin primers but no reverse transcriptase

NbTc1 transposase ORF. A single transcript of approximately 427 bp in length, consistent with the expected size of the *NbTc1* transposase transcript was detected (Fig. 5). In addition, our search of a *N. bombycis* EST database (286,518 *N. bombycis* ESTs) revealed two expressed sequence tags which matched 43 of 44 nucleotides of *NbTc1* in separate regions of the element (Table I), confirming that *NbTc1* shows transcriptional activity.

Discussion

Here we describe a new Tc1-like element, *NbTc1*, found in the microsporidian *N. bombycis*. While a number of retrotransposons have been described for many species of Microsporidia (Hinkle *et al*. 1997, Mittleider *et al*. 2002, Xu *et al*. 2006, Williams *et al*. 2008), few microsporidian DNA transposons have as yet been discovered (Williams *et al*. 2008). *NbTc1*, a complete DNA transposon which contains the D,D34D catalytic motif, is the first transcriptionally active DNA transposon to be identified in a microsporidian. Copies of *NbTc1* are widely distributed within the *N. bombycis* genome (10 out of 14 *N. bombycis* chromosomes contain *NbTc1* sequences) and exhibit a low level of sequence variation. The regions flanking the *NbTc1* elements show the TA dinucleotide target site duplication typical of Tc1/mariner elements, but no other strong insertion site specificity is seen. The only other known example of transcription of a transposable element in Microsporidia is the Ty3/Gypsy retrotransposon transcript found in an EST survey of *Edhazardia aedis* (Gill *et al*. 2008).

Due to their widespread distribution and apparent lack of requirement for host-specific transposition factors, transformation vectors based on Tc1/mariner elements have been useful in insertional mutagenesis studies in many organisms including protists, fish, insects and mammals (Gueiros-Filho and Beverley 1997, Schouten *et al*. 1998, Sherman *et al*. 1998, Adelman *et al*. 2002). *NbTc1*, the *N. bombycis* Tc1-like transposon described here, which shows transcriptional activity, sequence conservation, and a low degree of insertion site specificity, may have a number of valuable applications as a vector for studies in the Microsporidia.

Phylogenetic analysis indicates that the *NbTc1* transposon is not closely related to known Tc1 elements from other species, suggesting that it may belong to an unique Tc1 superfamily. LTR retrotransposons such as the Ty3/Gypsy retrotransposon in *N. bombycis* have been found in most Microsporidia (Xu *et al*. 2006), suggesting that these elements were present in the common ancestor of the Microsporidia. However, since few examples of Tc1 elements have been reported in the Microsporidia or the fungi, more data are needed before conclusions can be made regarding the evolutionary origin of the Tc1-like element in *Nosema bombycis*.

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References

Adelman Z.N., Jasinskiene N., Jame A.A. 2002. Development and applications of transgenesis in the yellow fever mosquito, *Aedes aegypti*. *Molecular and Biochemical Parasitology*, 121, 1–10. DOI: 10.1016/S0166-6851(02)00028-2.

Avancini R.M.P., Kimberley W.K.O., Robertson H.M. 1996. The genomes of most animals have multiple members of the Tc1 family of transposable elements. *Genetica*, 98, 131–140. DOI: 10.1007/BF00121361.

Daboussi M J., Langin T., Brygoo Y. 1992. Fot1, a new family of fungal transposable elements. *Molecular and General Genetics*, 232, 12–16. DOI: 10.1007/BF00299131.

Emmons S.W., Yesner L., Ruan K.S., Katzenberg D. 1983. Evidence for a transposon in *Caenorhabditis elegans*. *Cell*, 32, 55–65. DOI: 10.1016/0092-8674(83)90496-8.

Franz G., Savakis C.1991.Minos, a new transposable element from *Drosophila hydei*, is a member of the Tc1-like family of transposons. *Nucleic Acids Research*, 19, 6646. DOI: 10.1093/nar/19.23.6646.

Gill E.E., Becnel J.J., Fast N.M. 2008. ESTs from the microsporidian *Edhazardia aedis*. *BMC Genomics*, 9, 296. DOI: 10.1186/1471-2164-9-296.

Gueiros-Filho F.J., Beverley S.M. 1997. Trans-kingdom transposition of the *Drosophila* element mariner within the protozoan *Leishmania*. *Science*, 276, 1716–1719. DOI: 10.1126/science.276.5319.1716.

Hinkle G., Morrison H.G., Sogin M.L. 1997. Genes coding for reverse transcriptase, DNA-directed RNA polymerase, and chitin synthase from the microsporidian *Spraguea lophii*. *Biological Bulletin*, 193, 250–251.

Hirt R.P., Logsdon J.M., Healy B., Dorey M.W., Doolittle W.F., Embley T.M. 1999. Microsporidia are related to fungi: Evidence from the largest subunit of RNA polymerase II and other proteins. *Proceedings of the National Academy of Sciences*, 96, 580–585. DOI: 10.1073/pnas.96.2.580.

Ivics Z., Hackett P.B., Plaster R.H., Izsvak Z.1997. Molecular reconstruction of Sleeping Beauty, a Tc1-like transposon from fish and its transposition in human cells. *Cell*, 91, 501–510. DOI: 10.1016/S0092-8674(00)80436-5.

Izsvak Z., Ivics Z., Plasterk R.H. 2000.Sleeping Beauty, a wide host-range transposon vector for genetic transformation in vertebrates. *Journal of Molecular Biology*, 302, 93–102. DOI: 10.1006/jmbi.2000.4047.

James T.Y., Kauff F., Schoch C.L., Matheny P.B., Hofstetter V., Cox C.J., Celio G., Gueidan C., Fraker E., Miadlikowska J., Lumbsch H.T., Rauhut A., Reeb V., Arnold A.E., Amtoft A., Stajich J.E., Hosaka K., Sung G.H., Johnson D., O'Rourke B., Crockett M., Binder M., Curtis J.M., Slot J.C., Wang Z., Wilson A.W., Schmitt I., Schultz M., Yahr R., Hibbett D.S., Lutzoni F., McLaughlin D.J., Spatafora J.W., Vilgalys R. 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature*, 443, 818–822. DOI: 10.1038/nature05110.

Keane T.M., Creevey C.J., Pentony M.M., Naughton T.J., McInerney J.O. 2006. Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified. *BMC Evolutionary Biology*, 6, 29. DOI: 10.1186/1471-2148-6-29.

Keeling P.J. 2003. Congruent evidence from alpha-tubulin and beta-tubulin gene phylogenies for a zygomycete origin of mi-

crosporidia. *Fungal Genetics and Biology*, 38, 298–309. DOI: 10.1016/ S1087-1845(02)00537-6.

Keeling P.J., Luke M.A., Palmer J.D. 2000. Evidence from beta-tubulin phylogeny that microsporidia evolved from within the fungi. *Molecular Biology and Evolution*, 17, 23–31.

Langin T., Capy P., Daboussi M.J. 1995. The transposable element impala, a fungal member of the Tc1-mariner superfamily. *Molecular and General Genetics*, 246, 19–28. DOI: 10.1007/ BF00290129.

Lee S.C., Corradi N., Byrnes E.J., Torres-Martinez S., Dietrich F.S., Keeling P.J., Heitman J. 2008. Microsporidia evolved from ancestral sexual fungi. *Current Biology*, 18, 1–5. DOI:10. 1016/j. cub.2008.09.030.

Li F., Wang Y. 2006. The Comparison of DNA Extraction Methods of Microsporidia. *Guangdong Sericulture*, 40, 32–34.

Liao L.W., Rosenzweig B., Hirsh D. 1983. Analysis of a transposable element in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences*, 80, 3585–3589. DOI: 10.1073/ pnas.80.12.3585.

Mittleider D., Green L.C., Mann V.H., Michael S.F., Didier E.S., Brindley P.J. 2002. Sequence survey of the genome of the opportunistic microsporidian pathogen, *Vittaforma corneae*. *Journal of Eukaryotic Microbiology*, 49, 393–401. DOI: 10.1111/j.1550-7408.2002.tb00218.x.

Plasterk R.H.A., Luenen H.G.A.M. 2002. The Tc1/Mariner family of transposable elements. In: (Eds. N.L. Craig, R. Craigie, M. Geller and A.M. Lambowitz) *Mobile DNA II*. ASM Press, Washington DC, 519–532.

Robertson H.M. 2002. Evolution of DNA transposons in eukaryotes. In: (Eds. N.L. Craig, R. Craigie, M. Geller and A.M. Lambowitz) *Mobile DNA II*. ASM Press, Washington DC, 1093–1110.

Rosenzweig B., Liao L.W., Hirsh D. 1983. Sequence of the *C. elegans* transposable element Tc1. *Nucleic Acids Research*, 11, 4201–4209. DOI: 10.1093/nar/11.20.7137.

Schmidt H.A., Strimmer K., Vingron M., Haeseler A. 2002. TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. *Bioinformatics*, 18, 502–504. DOI: 10.1093/bioinformatics/18.3.502.

Schouten G.J., Luenen V.H., Verra N., Valerio D., Plasterk R. 1998. Transposon Tc1 of the nematode *Caenorhabditis elegans* jumps in human cells. *Nucleic Acids Research*, 26, 3013–3017. DOI: 10.1093/nar/26.12.3013.

Shao H., Tu Z. 2001. Expanding the diversity of the IS630-Tc1-mariner superfamily: discovery of a unique DD37E transposon and reclassification of the DD37D and DD39D transposons. *Genetics*, 159, 1103–1115.

Sherman A.A., Dawson C., Mather H., Gilhooley Y., Li R., Mitchell D.F., Sang H. 1998. Transposition of the *Drosophila* element mariner into the chicken germ line. *Nature Biotechnology*, 16, 1050–1053. DOI: 10.1038/3497.

Silva J.C., Bastida F., Bidwell S.L., Johnson P.J., Carlton J.M. 2005. A potentially functional mariner transposable element in the protist *Trichomonas vaginalis*. *Molecular Biology and Evolution*, 22, 126–134. DOI: 10.1093/molbev/msh260.

Starr T.K., Largaespada D.A. 2005. Cancer gene discovery using the Sleeping Beauty transposon. *Cell Cycle*, 4, 1744–1748.

Tamura K., Dudley J., Nei M., Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599. DOI: 10.1093/molbev/msm092.

Thomarat F., Vivares C.P., Gouy M. 2004. Phylogenetic analysis of the complete genome sequence of *Encephalitozoon cuniculi* supports the fungal origin of microsporidia and reveals a high frequency of fastevolving genes. *Journal of Molecular Evolution*, 59, 780–791. DOI: 10.1007/s00239-004-2673-0.

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F., Higgins D.G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 25, 4876–4882. DOI: 10.1093/nar/25.24.4876.

Williams B.A.P., Lee R.C., Becnel J.J., Weiss L.M., Fast N.M., Keeling P.J. 2008. Genome sequence surveys of *Brachiola algerae* and *Edhazardia aedis* reveal microsporidia with low gene densities. *BMC Genomics*, 9, 200. DOI: 10.1186/1471-2164-9-200.

Wittner M. 1999. Historic perspective on the microsporidia: expanding horizons. In: (Eds. M. Wittner and L.M. Weiss) *The Microsporidia and Microsporidiosis*. ASM Press, Washington DC, 1–6.

Xu J.S., Pan G.Q., Li T., Zhou Z.Y., Xiang Z.H. 2006. The varying microsporidian genome: existence of LTR retrotransposon in domesticated silkworm parasite *Nosema bombycis*. *International Journal for Parasitology*, 36, 1049–1056. DOI:10. 1016/j.ijpara.2006.04.010.

Zagoraiou L., Drabek D., Alexaki S., Guy J.A., Klinakis A.G., Langeveld A., Skavdis G., Mamalaki C., Grosveld F., Savakis C. 2001. *In vivo* transposition of Minos, a *Drosophila* mobile element, in mammalian tissues. *Proceedings of the National Academy of Sciences*, 98, 11474–11478.

Zwaal R.R., Broeks A., Vanmeurs J., Groenen J., Plasterk R. 1993. Target-selected gene inactivation in *Caenorhabditis elegans* by using a frozen transposon insertion mutant bank. *Proceedings of the National Academy of Sciences*, 90, 7431–7435. DOI: 10.1073/pnas.90.16.7431.

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