The Chitinase Gene of the Silkworm, *Bombyx mori*, Contains a Novel Tc-like Transposable Element*

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We have determined the cDNA sequence and the genomic organization of the chitinase gene of the silkworm, *Bombyx mori*. The cDNA encodes 544 amino acids having 83% amino acid homology to the chitinase of the tobacco hornworm, *Manduca sexta*. The total length of the gene is larger than 25 kilobase pairs, and it is separated into 11 exons. The intron-exon boundaries are all in accordance with the GT-AG rule. Also, the TATA box sequence was found in the 5′ upstream region of the gene, and the gene is mapped on the seventh chromosome. A novel DNA type transposon that shows similarity to the Tc-like element was found in the third intron in some strains of *B. mori*; other strains, however, lack this element in the same intron. This element has long terminal inverted repeats, presumably encodes a transposase of about 340 amino acids with a DDE motif, and has an amino-terminal-domain with a strong nuclear localization function. Seven other transposable elements with homologous but distinct sequences were isolated from the *B. mori* genome. Together with plaque hybridization results, our findings suggest that these novel elements exist in multiple copies constituting a new Tc-like transposable element family in the silkworm genome.

Chitin is a β(1,4)-linked polymer of N-acetylglucosamine believed to be the second largest bio-mass next to cellulose. Insects utilize it as the major component of their exoskeleton. When bound to proteins, chitin is strong enough both mechanically and biochemically to protect and light enough to allow smooth locomotion. However, once made as a component of the integument, chitin, unlike bone in vertebrates, has no growth capability, and the insects have to molt or undergo metamorphosis to reconstruct their integuments. Insect chitinase (family number 18 of glycosyl hydrolases, endochitinase) is induced by ecdysteroids at the time of molting and metamorphosis of the larvae to degrade most of the older chitin (1, 2). Further hydrolysis of the partially digested chitin is done by β1,4-N-acetylgalactosaminidase (exochitinase) that is also inducible by ecdysteroid (3). The recycling of β1,4-N-acetylgalactosamine from the older integument to the new integument was shown in larval-adult molting of Locusta migratoria (4) and larval-larval molting of Drosophila melanogaster (5). From insects, the chitinase cDNA of the tobacco hornworm *Manduca sexta*, has been isolated (6). With more than 4000 years of domestication history (7), *Bombyx mori* is one of the genetically most well studied organisms and is also a suitable model for hormone research. The existence of the molting hormone secreted from the prothoracic gland was experimentally shown using *B. mori* pupae (8, 9). The molting hormone, ecdysone, was isolated from the pupae of *B. mori* and crystallized, and its chemical structure was determined (10). The existence of the neuropeptide called prothoracicotropic hormone that stimulates the release of ecdysone was predicted (11), and the hormone was finally isolated and characterized (12).

Here we describe the cDNA cloning, characterization of the genomic organization, and chromosomal localization of the *B. mori* chitinase gene. Moreover, we have shown the presence of the novel Tc-like transposable element in the intron of this gene in a strain-dependent manner. The distribution and function of this *Tc* element transposable family are discussed.

EXPERIMENTAL PROCEDURES

*Insects and Genomic DNA Isolation—*The larvae of *B. mori* strain Shunrei X Showgetau were purchased from Kanebo Silk Elegance Co. (Kasugai, Japan). High molecular weight genomic DNA of other *B. mori* strains and insects was prepared as described previously (13).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR),† Rapid Amplification of 5′ Ends of cDNA (5′-RACE), and Rapid Amplification of 3′ Ends of cDNA (3′-RACE)—Total RNA of *B. mori* Shunrei X Showgetau strain was isolated from the integument of a prepupa (14), and the first-strand cDNA was synthesized. Degenerative forward primers F1 (5′-GCCA/GTNGT/CAT/TGATGGTT/CTAT-3′), F2 (5′-GA/TGAT/CAT/CCGGC/NAAA/GAAA/GTG-3′), and F3 (5′-GAA/GT/CA/GTNGAT/CATAA/GAAA/GTA-3′) and reverse primers R1 (5′-GGA/GCGAC/TCCGTAAT/TGATGGTT/CTAT-3′), R2 (5′-CA/GTTCTCA/TGATGA/GTNGAT/CATAA/GAAA/GTA-3′), R3 (5′-TCCA/CTTCGAT/TGATGA/GTNGAT/CATAA/GAAA/GTA-3′), R4 (5′-TCCA/CTTCGAT/TGATGA/GTNGAT/CATAA/GAAA/GTA-3′), and R5 (5′-TCCA/CTTCGAT/TGATGA/GTNGAT/CATAA/GAAA/GTA-3′) were designed from the amino acid sequence of the chitinase of *M. sexta* (Ref. 6; GenBank™ accession number U002270). The amino acid positions were chosen to minimize the degeneracy. Amplification reactions (100 μl) contained 2 μl of first-strand cDNA, 0.2 μM degenerative forward primer, 10 μM degenerative reverse primer, 1.25 μl Taq polymerase (Promega), 0.5 μM degenerative forward primer, 10 μM degenerative reverse primer, 5 μM primers, 1× Pwo buffer, 200 μM dNTPs, and 2 μl of cDNA in a total reaction volume of 50 μl. The PCR was performed in a thermal cycler (Perkin Elmer) using a touchdown PCR program with touchdown temperature of 60°C.

† The abbreviations used are: RT-PCR, reverse transcription-polymerase chain reaction; PCR, polymerase chain reaction; 5′-RACE, rapid amplification of 5′ ends of cDNA; 3′-RACE, rapid amplification of 3′ ends of cDNA; RAPD, random amplified polymorphic DNA; BmTc1, *Bombyx mori* Tc-like element 1; GFP, green fluorescent protein; kb, kilobase pair(s); Mb, megabase pair(s); EST, expressed sequence tag.
verse primer, and 5 units of Ex Taq DNA polymerase (Takara). Temperature cycling was carried out at 94 °C for 3.5 min, followed by 32 cycles of 1.5 min at 94 °C, 2 min at 45 °C, and 3 min at 72 °C, and an additional 12 min at 72 °C. A combination of nested primers (5′-GGCG-ACCATAAGCTTGAGAT-3′ and 5′-CCGCTCAATACAGTCGTTG-3′) or (5′-TACCTCTTATCTACTGTCCTG-3′) was used for 5′-RACE and 3′-RACE, respectively. The full length B. mori chitinase cDNA amplification reaction (50 μl) contained 1 μl of B. mori first-strand cDNA, 0.3 μM primer B1 (5′-GGCGACTCTG-CAGCGAAGACC-3′) and primer B2 (5′-GACCACTCTAGTTGCCC-CT-3′), and 2.6 units of Expand High Fidelity System Enzyme (Boehringer). Temperature cycling was carried out at 94 °C for 2 min, followed by 25 cycles of 30 s at 94 °C, 30 s at 94 °C, 30 s at 55 °C, and 2 min at 72 °C and an additional 9 min 72 °C.

DNA Sequencing—PCR products were cloned into pCRII vector (Invitrogen) or pGEMEasy vector (Promega) and sequenced by using the dye terminator method. Gel analysis was done at Cybergene (Novum, Huddinge, Sweden).

Screening of the Lambda Phage Genomic Library—from the EMBL3 B. mori genomic DNA library (15), clones containing the chitinase gene were isolated with the 32P-labeled 0.64-kb PCR fragment (probe 1) generated from B. mori chitinase cDNA with primers 5′-GGCGAATGGTTGCACCTG-3′ and 5′-TACGCATACAAGGGAACTCA-3′. The deduced amino acid sequences of the 0.5-kb fragment of B. mori chitinase gene intron 1, was used to isolate the promoter region-containing clones. Hybridizations of the plaque-blotted Hybond N+ membranes (Amersham Pharmacia Biotech) were performed in 5× SSC (750 mM NaCl, 75 mM sodium citrate, pH 7.0), 5× Denhardt’s solution, and 0.5% SDS at 65 °C overnight with probe 1 or probe 2. The membranes hybridized with probe 1 were washed twice in 2× SSC, 0.5% SDS for 15 min at 65 °C, and washed more than twice with 0.2× SSC, 0.2% SDS for 15 min at 65 °C until the background signals were reduced. The membranes hybridized with probe 2 were washed under less stringent conditions.

Sequence Analysis—The finished DNA sequence was analyzed with DNA STAR and DNA Strider programs. BLASTN and BLASTP programs were used to find similarities to sequences in public databases. The promoter sequence was analyzed with the TSS program. Alignment of Te/meriner transposases was done by using the GCG program.

Determination of the B. mori Chitinase Gene Structure—All the intron/exon junctions of the B. mori chitinase gene were determined by sequencing the genomic clones. The sizes of introns were determined by PCR or sequencing. The existence of the B. mori Te-like element 1 (BmTe1) within intron 3 was checked by PCR with primers B3 (5′-CA-GTATTTGGCGGTGTACCAGGC-3′) and B4 (5′-GGGTGTTGGAAC-GGAGGATATGGG-3′).

Analysis of BmTe Elements—BmTe family elements were isolated from the genome of B. mori by PCR with the primers T1 (5′-TACCTCTTCTTATCTACTGTCCTG-3′) or T2 (5′-TACCTCTTCTTATCTACTGTCCTG-3′). Amplification conditions were essentially the same as those for full-length chitinase cDNA, using 0.2 μg of B. mori genome DNA as a template. 32P-labeled BmTe probes generated by PCR with primers 5′-GACTCAAGCTCTGAGCTCATCC-3′ and 5′-ATAGCTGAGGTTATATGCTGTA-3′ were used for the genomic Southern and plaque hybridization in essentially the same way described for the chitinase gene screening.

Linkage Mapping of the B. mori Chitinase Gene—Linkage mapping was done by analyzing the genetic segregation of 95 individuals of F2 generated from a crossover between the p50 and C108 strains. B3 and B4 primers were used for amplification of the fragments. Random amplified polymorphic DNA (RAPD) markers were used to compare the segregation pattern with these chitinase marker fragments (13).

Subcellular Localization Analysis of Amino-terminal Domain Encoded by BmTe1—The BmTe1 DNA region encoding the amino-terminal 68 amino acids was PCR-amplified with primers 5′-AGATTAATCACACCATGAGAGAGGAGAC-3′ and 5′-AGATTAATCACACCATGAGAGAGGAGAC-3′ and ligated into the EcoRI site of pEGFP-C2 vector to construct pEGFP-C2-BmTe1N1. COS-7 cells maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and 25 μg/ml kanamycin were plated onto 6-well plates (Costar) 48 h before transfection and transfected with 0.5 μg of pEGFP-C2-BmTe1N1 or pEGFP-C2 in each well using Fugene 6 (Roche Molecular Biochemicals). 30 h after transfection, cells were observed through fluorescent confocal microscopy (Leica).

RESULTS

PCR Amplification of the Chitinase cDNA of B. mori and the Deduced Amino Acid Sequence—A set of nested degenerative primers (Fig. 1A) was used to PCR-amplify B. mori chitinase cDNA (Fig. 1B). The longest PCR fragment (Fig. 1B, lane 4) was sequenced, and the deduced amino acid sequence shows homology to the corresponding sequence of M. sexta chitinase. 5′-RACE and 3′-RACE were done with primers designed from the DNA sequences of this RT-PCR product (Fig. 1, C and D). The deduced amino acid sequences of the 0.5-kb fragment of 5′-RACE and the 1.8-kb fragment of 3′-RACE show homology to M. sexta chitinase, and the latter contains poly(A) signal before the poly(A) sequence. B1 and B2 primers were designed,
FIG. 2. The cDNA sequence and the deduced amino acid sequence of B. mori chitinase. The cDNA sequence (Genbank™ accession number AF273695) is indicated on the top line, and the deduced amino acid sequence is indicated on the second line. Intron insertion sites are indicated by black triangles. The 20-amino acid sequence matching the amino-terminal end of B. mori chitinase is underlined. The putative active site is boxed.
and the 1.7-kb cDNA encoding full-length \textit{B. mori} chitinase was amplified. The open reading frame contains the AD-SRARIVCFSINWAYRP motif after the putative signal peptide sequence (Fig. 2). This motif matches the reported amino-terminal sequence of the chitinase purified from \textit{B. mori} larvae (16). As deduced from the cDNA sequence, the total length of \textit{B. mori} chitinase was 544 amino acids and showed 83\% homology to \textit{M. sexta} chitinase. The hydrophilicity plots were quite similar between these two variants of chitinase. The putative active site region FDGLDLDWEYP, conserved in chitinases of holometabolous insects (23), was also conserved in \textit{B. mori} chitinase (Fig. 2). At the nucleotide level, the coding region of the two insect chitinases showed 72\% homology.

**Genomic Structure of the Chitinase Gene of \textit{B. mori}**—We screened the \textit{B. mori} genomic phage library with cDNA probe 1, and two clones covering the whole chitinase gene except for the putative active site region were identified. The entire genomic sequence of the two insect chitinases showed 72\% homology.

The genomic organization of the \textit{B. mori} chitinase gene. 11 exons (E1 to E11) are represented by boxes, and 10 introns (I1 to I10) are indicated by lines. Amino acid-encoding regions of exons are shown as black boxes. The translation initiation site (ATG) is indicated. The \textit{BmTc1} element is shown as a box with an arrow showing the transcription direction of a putative open reading frame.

![Figure 3](https://example.com/figure3.png)

**Fig. 3. The genomic organization of the \textit{B. mori} chitinase gene.** 11 exons (E1 to E11) are represented by boxes, and 10 introns (I1 to I10) are indicated by lines. Amino acid-encoding regions of exons are shown as black boxes. The translation initiation site (ATG) is indicated. The \textit{BmTc1} element is shown as a box with an arrow showing the transcription direction of a putative open reading frame.

**Table 1**

Sequences of the intron/exon junctions of the chitinase gene of \textit{Bombyx mori}

| Exon   | Sequence                                                                 | Location |
|--------|---------------------------------------------------------------------------|----------|
| 1      | ACAGACGCTAACC                                                             | Exon 1   |
| 2      | TTA GTG CAG TGT G                                                          | Exon 2   |
| 3      | T ATG CAT CCT GAG                                                          | Exon 3   |
| 4      | A GA A AGC GTG GTC                                                         | Exon 4   |
| 5      | CA GAG CTG TGT CA                                                         | Exon 5   |
| 6      | G AAA CTT AAC GTG                                                         | Exon 6   |
| 7      | G CTT TAT TAT GGA                                                         | Exon 7   |
| 8      | C GCA ACT CCT ACT                                                         | Exon 8   |
| 9      | G GAA CCA CCC ACG                                                         | Exon 9   |
| 10     | A GAG TGT AGG AGG                                                         | Exon 10  |
| 11     |                                                                                         | Exon 11  |

**Fig. 4. Putative promoter sequence of the \textit{B. mori} chitinase gene (GenBank\textsuperscript{TM} accession number AF273702).** The TATA box motif is underlined. The predicted Sp1 binding sites are indicated by broken lines. The 5' end sequences found in the chitinase cDNA (Fig. 2) are shown in bold.

By PCR with B3 and B4 primers designed to amplify intron 3 of the chitinase gene, homozygotic and heterozygotic existence of the element within this intron was suggested in the C108 and Kokin strain of \textit{B. mori}, respectively. For five other strains (p50, J137, Oha, Sekko, and Kansen), no insertion of the \textit{BmTc1} element was suggested. An additional TATA motif in the putative insertional position of the element was observed in the genes of four strains lacking \textit{BmTc1}.

**Table 1**

Sequences of the intron/exon junctions of the chitinase gene of \textit{Bombyx mori}
Using primer T1 or T2 designed from the terminal sequences of the BmTc1 element, we have PCR-amplified fragments ranging from 1.2 to 1.6 kb from the template DNA of both the C108 and p50 strains. The 1.6-kb fragments amplified from C108 strain DNA with primer T1 were subcloned. The HaeIII digestion pattern of the insert DNA of each clone suggested the existence of the BmTc family (data not shown). DNA sequences of seven clones were determined and found to be homologous, constituting a new Tc-like element family in B. mori (Fig. 7).

Using T1 primers, 1.5-kb PCR amplification fragment was obtained from the DNA of B. mandarina. However, DNA of other silk moths (Samia cynthia ricini, Antheraea yamamai, and Dictyoploca japonica), D. melanogaster, H. sapiens, and Rattus norvegicus gave no amplification products.

The copy number of the BmTc family is estimated as 43 per haploid B. mori genome because the genome size is 530 Mb (26, 27), and 40.5 positive plaques were identified on a membrane containing a total of a 500-Mb insert of B. mori DNA by hybridizing with BmTc1 probe. The strain-specific genomic Southern hybridization patterns were observed with BmTc1 probe, suggesting that BmTc family elements are mostly integrated into different part of the genome of the two B. mori strains (Fig. 8).

We also tested the nuclear localization activity of the amino-terminal 68 amino acids of BmTc1 putative transposase (BmTc1N1) by fusion to GFP. COS-7 cells were transfected with pEGFP-C2-BmTc1N1 that could express the GFP-BmTc1N1 protein. In these cells, apparent nuclear localization of GFP-BmTc1N1 was observed in more than 90% of the transfected cells (Fig. 9, A and B).
alone displayed a diffuse and uniform cellular fluorescence (Fig. 9, C and D).

Genome Mapping of the Chitinase Gene of B. mori—Chromosomal localization of the chitinase gene was determined by linkage analysis with RAPD markers mapped on each chromosome using polymorphism between the C108 and p50 strains (13). Primers B3 and B4 were used to detect segregation of F2 progenies resulting from a cross between p50 and C108. RAPD marker R1.41 showed cosegregation with the chitinase gene (Table II). The recombination value was calculated as follows: \[ ((2+2+2+4)/95) \times 2 = 21.05\% \] (multiplied by 2 due to lack of crossover in female B. mori).

The R1.41 marker was previously mapped on chromosome 7 of the B. mori genome, and we conclude that the chitinase gene is also mapped on this chromosome.

**DISCUSSION**

Chitinase cDNA has been cloned from various organisms including microorganisms, plants, and higher animals. Chitinase has three major functional roles, controlling growth via degradation of chitin in the organism. In insects (28) and yeasts (29), the enzymes play essential roles in the regulation of growth. In plants, chitinases are induced by wounding and thought to be involved in protection from fungal infection. On the other hand, insect pathogens such as baculovirus have the chitinase gene within their genome and use this enzyme for the invasion of insect bodies (30). We have mapped the chitinase gene of B. mori to chromosome 7; interestingly, the fungal resistance gene called cal has been mapped on the same chromosome (31). The cDNA (17) and the genomic gene (19) of human chitinase have been cloned recently. The expression of human chitinase is induced in activated macrophages, and this enzyme might be related to the defense mechanism against fungal infections. An increase in chitinase activity was also observed in the spleen of guinea pig after intravenous infection with the pathogenic fungus *Aspergillus fumigatus* (32).

We found a published cDNA encoding a putative chitinase of B. mori similar to our sequence (Ref. 33; GenBank accession number U86876). The amino-terminal regions of the two cDNA sequences were almost identical; however, they differ in the carboxyl-terminal coding region. We have found direct repeats of 112 base pairs in the cDNA sequence published previously (33). This second repeat is inserted 5 base pairs before the stop codon of our sequence and encodes 22 additional amino acids. We could not find this second repeat sequence in the B. mori chitinase gene. Also, there was no full-length cDNA clone identical to the sequence of U86876 in the clones obtained by...
Fig. 8. Southern hybridization of the genomic DNA of two different B. mori strains with 32P-labeled BmTc1 element. B. mori genomic DNA (5 μg) was digested and blotted onto positively charged Nylon membrane, hybridized, and washed under low stringency conditions. BPB and XC denote bromphenol blue and xylene cyanole dye markers, respectively.

Fig. 9. Amino-terminal domain of BmTc1 putative transposase contains nuclear localization activities. COS-7 cells were transfected with pEGFP-C2-BmTc1N1 (A and B) and pEGFP-C2 (C and D) to express GFP-BmTc1N1 (the amino-terminal 68 amino acids) and GFP, respectively, and the GFP green fluorescence of transfected cells was imaged using confocal fluorescence microscopy. Representative cells are shown.

RT-PCR, although the primers used in the experiment should also amplify the cDNA. The chitinase amino acid sequence reported here contains the amino-terminal 20-amino acid sequence ADSRARIVCYFSNWAVYRPG, matching the sequence determined as the amino-terminal sequence of the purified chitinase of B. mori. However, the deduced amino acid sequence of U86876 contains S instead of A at the first position of this motif. We need to further investigate the reason for the difference between the two chitinase cDNA sequences. One possibility is that the discrepancies between the cDNAs are related to strain differences, and another possibility is that the direct repeat sequence in the cDNA might be regulated at the splicing level. The copy number of the chitinase gene of B. mori is probably 1 per haploid genome. However, in D. melanogaster and Aedes aegypti, at least 4 chitinase genes are found (34). It is likely that the original copy number of the chitinase gene of Arthropods was 1, and in Diptera, the gene was amplified, whereas in Lepidoptera, the gene was not amplified. However, we may need more detailed analysis, e.g., the gene targeting method recently developed in D. melanogaster (35) to knock out the gene, to draw a conclusion on the copy number of the chitinase gene and the functional role of its products in the insect.

Interestingly, expression of M. sexta chitinase gene was strongly induced in a few tissues of insect larvae including the epidermis, foregut, and hindgut (6). The molecular mechanism of this tissue-specific hormone-responsive induction of insect chitinase has not been studied because the cloning of the 5′ promoter region of the gene was unsuccessful (36). In this study, we have cloned a 5-kb promoter region of the B. mori chitinase gene, and it would facilitate further study of the tissue-specific hormone-dependent expression of this gene.

The existence of the BmTc1 element in intron 3 might affect the splicing efficiencies of chitinase mRNA because it may form a stem loop structure by hybridizing at the long terminal inverted repeats. However, the molting process does not differ obviously between B. mori strains, and the physiological effects of BmTc1 insertion might be minimal. Heterogenic existence of BmTc1 in B. mori strains could support this conclusion.

Transposable elements are found in the genome of various organisms and believed to contribute to the reorganization of the genome through their vertical and horizontal transmission. An additional TATA sequence found in the third intron of the chitinase gene of some B. mori strains clearly suggests this model in higher organisms. Transposable elements are classified into two groups: (a) class I is retrotransposon-type elements that transpose via RNA intermediates, and (b) class II is DNA-type elements that transpose via DNA intermediates (21). Tc-mariner-like transposable elements represent one of the major superfamilies of the class II transposons. The trans-

Table II

| Chitinase | R1.41 | No. of individuals |
|----------|-------|--------------------|
| H        | H     | 42                 |
| P        | P     | 27                 |
| C        | C     | 16                 |
| C        | H     | 2                  |
| H        | C     | 2                  |
| H        | P     | 2                  |
| P        | H     | 4                  |
| P        | C     | 0                  |
possible element we found should belong to the Tc family, having a DDE motif in its putative transposase-encoding region. Another type of class II transposable element, Bmmar1, is also found in the genome of B. mori (37). Bmmar1 elements have a DDD motif and are classified as members of the mariner family. The estimated copy number of Bmmar1 is more than 1000 per haploid genome. On the other hand, we estimate the copy number of BmTc to be less than 50 per haploid genome. From the B. mori EST database, we have also found two clones (GenBank™ accession numbers AU004038 and AU004047) that have 88% and 89% nucleotide identities to the BmTc transposase-encoding region.

In this study, nuclear localization activity of the DNA binding domain of BmTc1 putative transposase is shown (Fig. 9). EST clone (GenBank™ accession number AU004038) also contains an open reading frame encoding a homologous amino acid sequence (data not shown). Both BmTc1 and the EST clone possess stop codons in the 3' region of the DNA binding domain coding sequences. Protein products of BmTc1 and the EST clone might act as dominant negatives to moderately suppress BmTc-type transposase in the cell nucleus of B. mori.

Recently, transposon vectors have been created successfully by modifying the inactive Tc1-like elements found in Salmonid (38). B. mori is an economically important species and is reared easily and cheaply on artificial diets. Protein production in B. mori larvae using the baculovirus system (39) has been shown to be quite efficient. BmTc elements have several favorable characteristics as the gene transfer vector of B. mori. The copy numbers of this element in the genome of B. mori are not too high, and the insertional sites should not be harmful to the viability of this organism. Moreover, the safety of BmTc elements in the human has been tested, at least in part, through the history of silkworm breeding. BmTc elements could be suitable gene transfer vectors for generating transgenic B. mori and other organisms. We found TATA box sequences, CAAT box sequences, arthropod capsise sequences (TCAGT), and a putative open reading frame in most of the BmTc family members (data not shown). The BmTc elements we found have stop codons and missense mutations within their putative transposase-encoding region. We are now interested in the generation of a gene transfer vector based on the BmTc element family.

In summary, we have cloned B. mori cDNA encoding chitinase of 544 amino acids, determined the genome structure of this gene, and found a novel DNA-type transposon in an intron of this gene in some strains of B. mori. We have also suggested nuclear localization activity of the amino-terminal DNA binding domain of the putative transposase and wide distribution of the homologous transposable elements in the genome of B. mori.

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