생명과학회지
Korean J. Life Science, 8(3), 285-293, 1998

Identification of Mariner-Like Element(MLE) Gene from *Bombyx mori.*

Jin-Sung Lee, Jae-Sam Hwang*, Yong-Sung Kim, Dong-Sang Suh** and O-Yu Kwon†***

Genome Center, Korea Research Institute of Bioscience & Biotechnology, KIST, Taejon 305-600, Korea
**National Sericulture & Entomology Research, R.D.A., Suwon 441-100, Korea*
***Dept. of Genetic Engineering, Sungkyunkwan Univ., Suwon 440-746, Korea*
****Dept. of Anatomy, Coll. of Medicine, Chungnam Natl. Univ., Taejon 301-131, Korea*

Abstract

We have cloned an internal fragment of the putative transposase gene of MLE in the silkworm, *Bombyx mori*, using PCR method with degenerative oligonucleotide primers designed to represent regions of amino acids encoding transposase. The resulting PCR clone, designed as BmoMAR, cords a partial ORF(152 a.a.) of MLE in which interrupted by five stop codons, and the sequence of its deduced amino acids showed 37% homology with *Mos*1, an active mariner, from *Drosophila mauritiana*. Furthermore, the BmoMAR exhibits nucleotide and amino acid homology with 59% and 37% from *Apis mellifera* and *D. mauritiana* 7.9 clone, respectively. This result strongly suggests that a MLE is present in the genome of *B. mori*.

Key words : *Bombyx mori. Mariner-like element(MLE), degenerative oligonucleotide primers*

Introduction

Transposable elements appear to be a common constituent of the eukaryotic genome[1,2]. In *Drosophila* several types of transposable element were identified well[3]. Among them, mariner element, 1.3 kbp, is a typical DNA intermediate transposon which include inverted 27-31bp terminal repeats and a single ORF(open reading frame) encoding a transposase[4]. According to the well-studied *Drosophila* P element[5], inverted terminal repeats serve as recognition site for the transposase and host proteins[6]. It is interesting considering the evolutional origin of mariner that mariner-like elements have been found in *pyathelminth*[7], a nematode[7], a centipede[8] and a mite[8], as well as throughout the class insecta[8,9].

Silkworm has been known as a commercially important insect as well as a honeybee. Advancing molecular biological methods of make it possible to understand insect physiology of silkworm, fruit fly and mosquito under molecular level. In particular, the technique of direct DNA-mediated transformation provides molecular breeding, and it helps to understand molecular physiology of insects. Although several attempts to transform silkworm have been reported[10,11], it has been not satisfied results to get a genetically transformed silkworm. Because there is no suitable vector for foreign DNA to introduce into silkworm's genome and to express as an exogenous protein. However, recently it is demonstrated that the mariner transposable element is capable of interplasmid transposition in the yellow fever mosquito[12,13],

† Corresponding author

and we suggest that MLE isolated from silkworm could be utilized to genetically transform the *Bombyx mori* as well as an useful universal vector for insect transformation, because of its broad taxanomic distribution and high homology in the insect species.

In this paper, we have examined the presence of mariner-like element in the genome of silkworm, *B. mori*, Using PCR technique, mariner-like element was amplified and determined its nucleotide sequence.

Materials and Methods

Genomic DNA Extraction

Genomic DNA was purified from the posterial silk grand of *Bombyx mori, Bombyx mandarina, Antheraea yamamai* and *Antheraea periny,* respectively. About 0.5 g of posterior silk gland was dissected from the larvae on 4th day of the final instar and washed with 1×SSC. The frozen silk gland in the nitroliquid was homogenized in a buffer of 50 mM Tris-HCl(pH 8.0), 100 mM NaCl, 10 mM EDTA, 1% SDS, 150 ug/ml proteinase K and incubated at 50℃ for 30 min. After extraction with phenol three times and two times of phenol/chloroform(1 : 1), the supernatant was precipitated with absolute cold EtOH. The purified DNA pellet was dissolved in TE buffer [10 mM Tris-HCl(pH 8.0), and 1 mM EDTA] containing RNase(10 ug/ml).

PCR conditions

On the basis of nucleotide sequences of mariner of *Drosophila mauritiana* and *Hyalophora cecropia.* one pair of degenerative primers(MAR124F ; 5'-tgggtnccncaygaryt-3', MAR275R ; 5'-attaaaagctgrtattcatc-3') were designed from the two conserved regions of amino acid sequence(WVPHEL and YSPDLAP) of transposase using a Applied Biosystems DNA Synthesizer(Medel 381A). PCR was performed on a Perkin-Elmer Cetus instrument. Standard PCR conditions consist of 35 cycles of denaturation at 96℃ for 1 min, and extension at 72℃ for 1 min. Initial denaturation before PCR cycles proceeded at 96℃ for 3 min, and then Taq DNA polymease(0.25 unit) was added. For each PCR, each of 40 ng of genomic DNA from four silkmoths was used in a 20 ul reaction volume. The PCR products were analyzed by agarose gel electrophoresis.

Cloning and sequencing

The PCR products were separated on 1% SeaPlaque agarose gel, and bands of the expected size were recovered using Gene Clean II kit(Bio 101, USA). The eluted fragments were directly ligated with pGemT vector(Promega, USA) and transformed into *E. coli* JM 109. This purified PCR clone was redissolved in the 30 ul of DW to give a concentration of 30-50 ng/ul. Fluorescent cycle sequencing was performed with 300-500 ng of this double stranded DNA in 20 ul sequencing reaction mix.

Sequence Data Analysis

Arrangement of sequences obtained from cloned DNAs were performed with the aid of a BLAST program with confirmation using Clustal W program[14]. Another program of DNASIS(Hitachi Software Engineering) was used for the sequence-conversion of nucleotide acid to amino acid. The translations were again aligned using Clustal W program with final manual adjustment.

Results

From four silkmoths, mariner-like element(MLE) was examined by PCR using degenerate primers. Figure 1a shows that a common intense fragment of about 0.5 kb was produced from *Bombux mori, Bombyx mandarina, Antheraea yamamai* and *Antheraea periny, respectively.* The sizes of amplified DNA were well corresponded to those so far reported MLE in other insects, and it is suggested that the amplified DNAs in this study originated from MLE of four silkmoths.

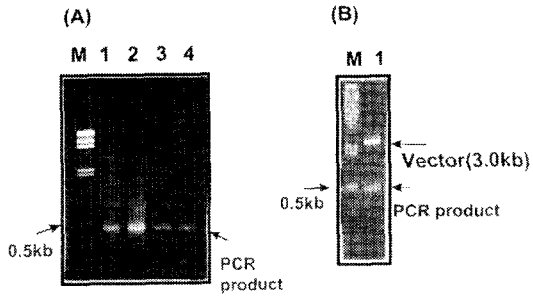

Fig. 1. PCR product amplified by degenerative primers from 4 silkmoths(A), and cloning of PCR product from Bombyx mori(B), On panel(A), (1) *Bombyx mori*; (2) *Bombyx mandarina*; (3) *Anthera yammai* and (4) *Anthera peryni, respectively, On panel(A)* : *(1) cloned PCR product, On both panels(A, B), M* ; *Molecular marker of* λ/HindIII.

```
TGGGTCCCACACGAGCTCACTGAAAGAGAGCTAATGAACCATGTACTCATTTGTGATTT
  W  V  P  H  E  L  T  E  R  E  L  M  N  H  V  L  I  C  D
TTTATTACGGCGTAATGAAACCGAACCATTTTTGAAGAAGCTGATAGCTGGTGATGAAA
F  L  L  R  R  N  E  T  E  P  F  L  K  K  L  I  A  G  D  E
AGTGGACCACGTACGACAAGAACGTGCGAAAAAGGTGGCGGTCAAAGGCCGGTCAGGCT
 K  W  T  T  Y  D  K  N  V  R  K  R  W  R  S  K  A  G  Q  A
TCACAGACTGGCGAATCCCGGGTTAACTCGCAACGAAGTGATGCTGTATGTGTAGTGGG
  S  Q  T  G  E  S  R  V  N  S  Q  R  S  D  A  V  C  V  V
ATGGGAAGGACATTATTCATTATTGAGCTTTTACCACCAGGCAGGCCTATCGATTCTGA
G  W  E  G  H  Y  S  L  L  S  F  Y  H  Q  A  G  L  S  I  L
ACTCTGCTGCGAACAACTGGTGAGATTAAAGCAAGAAGTCGAAATTAATCAACAGAAGG
 N  S  A  A  N  N  W  *  D  *  S  K  K  S  K  L  I  N  R  R
GGTGTGGTTTTTCATCACGATGACGCTAGACCTCACACATCTTTAGCCACTCAGTAAAA
  G  V  V  F  H  H  D  D  A  R  P  H  T  S  L  A  T  Q  *
ATTATGAGAGCTTGACTGGGATGTGTTAATGCATCCGCCGTATTGACCCGACCTCGCCC
K  L  *  E  L  D  W  D  V  L  M  H  P  P  Y  *  P  D  L  A
```

Fig. 2. Nucleotide and deduced amino acid sequences of the BmoMAR. The upper line represents the nucleotide sequence of BmoMAR and the lower line represents its translated amino acid sequence, respectively. Five asterisks in the amino acid sequence indicate stop codons.

To verify that those amplified DNA fragments were originated from transposase-encoding region of MLE, we examined the amplified DNA from *B. mori* and cloned. Several clones were tested for having amplified DNA. Figure 1b shows a recombinant DNA, named as BmoMAR, it was digested with *Eco*RI restriction endonuclease of multicloning site of pGemEasyT vector. The nucleotide sequences were determined from four clones of BmoMAR to exclude intrinsic error or mis-incorporation by *Taq* DNA polymerase itself. Figure 2 shows the nucleotide sequences and deduced amino acid sequences derived from four individual clones. A BmoMAR has 473 bp and 152 amino acid in length. But the amino acid sequence has five stop codons which is present at nucleotide position of 321(TGA), 327(TAA), 415 (TAA). 425(TGA) and 464(TGA), respectively.

The BmoMAR sequences were compared with these to Mos 1 element(DmMos 1) of *Drosophila mauritiana* (GeneBank accession no. X89923)(Figure 3a, 3b). The matching percentages were 53% at nucleotide level and positively 47% at amino acid level, respectively. Further comparison was performed with MLE families of eight different insect species so far reported on the nucleotide and amino acid sequence : *Hyalophora cecropia*(GeneBank accession no. L10443), *Haematobia irritans*(GeneBank accession no. L10473), *Oncopeltus fasciatus*(GeneBank accession no. L10490), *Drospphila mauritiana*(GeneBank accession no. X78906), *Cerotoma trifuricata*(GeneBank accession no. L10455l), *Chrysops vittatus*(GeneBank accession no. L10499), *Apis mellifera*(GeneBank accession no. L10433).

From overall pairwise-comparison except for BmoMAR, the matching percentages ranged from 41% to 73 % on the nucleotide level and from 14% to 65% on the amino acid level(Table 1), whereas the matching percentages of BmoMAR to each of MLE ranged from 43% to 59% and from 18% to 37%. This result indicates that the matching percentages of BmoMAR fall under the category of overall percentage. In particular, the highest homology with BmoMAR appeared from MLE of *Apis mellifera* at nucleotide level and MLE from *Drosophila mauritiana* 7.9 clone at amino acid level, respectively.

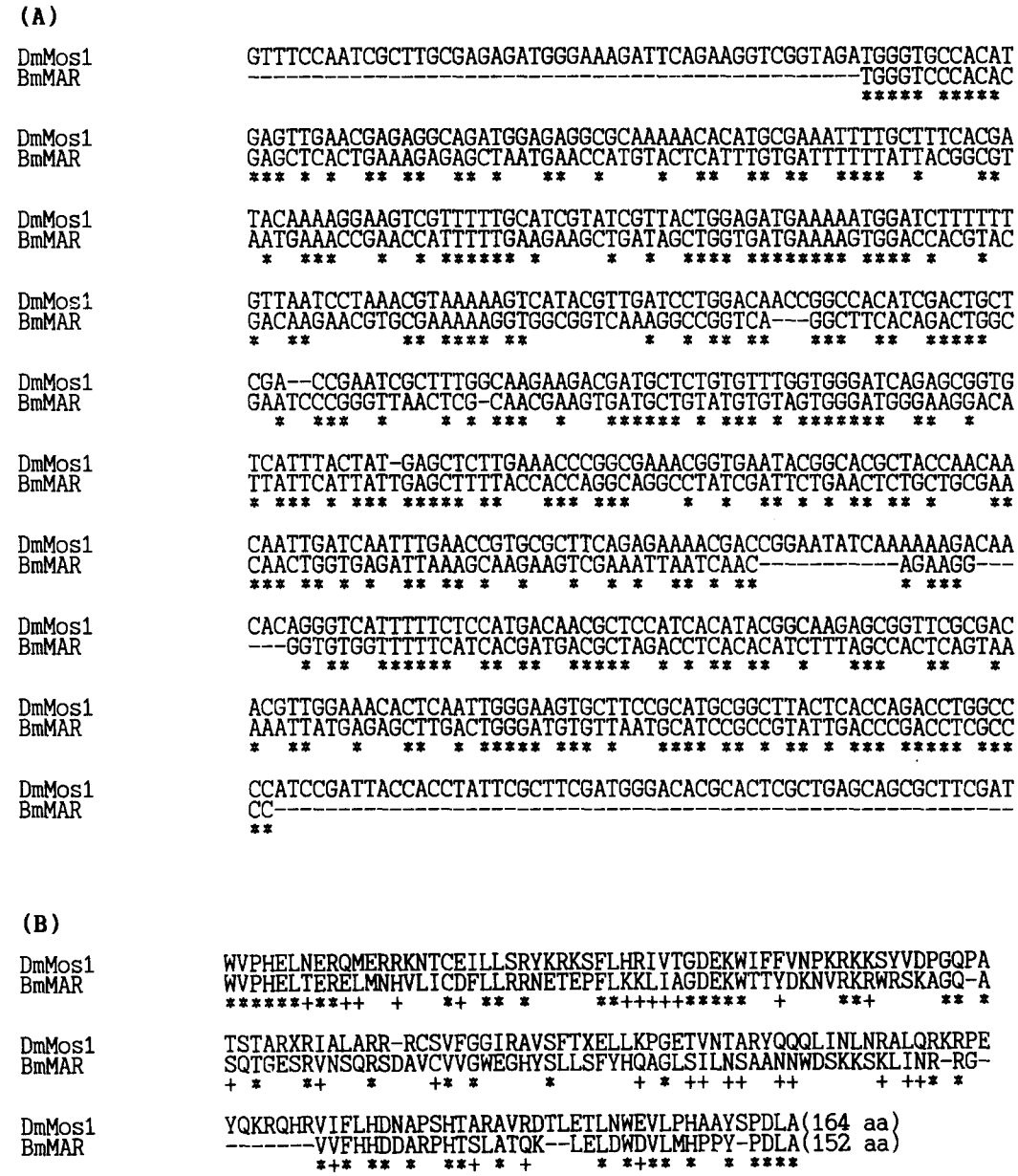

Fig. 3. Comparison of the nucleotide sequence(A) and deduced amino acid(B) of BmoMAR with those of D. *mauritiana's* Mos1. Nucleotide sequence of *D. mauritiana's* Mos1 is shown on upper line and its BmoMAR is on the lower line. Deleted regions are represented by hyphens in the both sequences(A, B). In(B), Identical and similar amino acid residues are indicated by asterisks and hyphens below, respectively.

Table 1. Identify of mariner sequences of nucleotide and amino acid among nine species. Abbreviations are defined in the Fig. 4. Figures in the upper part of the table are the number of DNA identifies, and those in the lower part are amino acid identifies.

Cvi	*Ctr*	*Fau*	*Hce*	*Ame*	*Hir*	*Ofa*	*Dma*	*Bmo*	*Cvi*
Cv		31	26	36	37	21	41	32	19
Ctr	43		25	29	22	14	28	28	18
Fa	52	46		25	24	63	26	28	19
H	46	42	47		65	23	55	35	21
A	53	44	49	48		19	57	38	19
H	46	73	44	44	46		17	19	18
Of	64	46	48	47	54	46		41	20
D	62	41	52	48	51	43	70		37
B	46	44	45	44	59	42	50	48	

Discussion

Mariner-like elements(MLE) were found in many other insects[8,9,15,16] as well as in other organisms[8,9,17], and were suggested to have spread horizontally in the genetic pool. On given our data, it was predicted that the genome of *Bombyx* genus might contain a MLE analog. With this in mind, the present study is to examine the presence of mariner in the genome of silkworm, B. *mori*. Our PCR result showed that the degenerative primers were bound to specific target template from four silkworm, and products amplified were very similar to those of them. It indicates that the conserved region of MLE transposase should be shared in the genome of four silkmoths, and the nucleotide sequence of BmoMAR derived from *B. mori* is shared with high homology with other insect's. Based on this result, this report is the first direct evidence that MLEs are commonly dispersed in genome of silkworms.

Lidholm[18] identified a mariner element which is inserted in an intron of the of the cecropin A gene of *H. cecropia*. The genome of this moth contains at least 1, 000 copies of mariner. Its sequence is 48% identical to the active *Mos* 1 element of *D. mauritiana*. Translation of its putative transposase gene required introduction of several deletions, which make frameshifts and make stop codons when it was aligned with that of the *Mos* 1 element. Sequences of BmoMAR show also similar results of Lidholm's[18] report that repeat stop codons present in the putative transposase in *Hyalophora cecropia*. This is suggested that BmoMAR is inactivated by insertion or deletion of its transposase. Interestingly, most reported mariner-like elements possessed also an ORF inactivated by insertion or deletion in their genomes. However, recently it is known that a selenoprotein has UGA codon in the ORF which conventionally only serves as a terminal signal, actually the protein translated is using UGA codon for the selenocysteine not for the stop codon[19]. From those information, we suggest that BmoMAR is a kind of pseudogene or a selenoprotein, which is one of the mariner-like elements present in *B. mori*.

The widespread but apparently sporadic distribution of MLE suggests that it might be developed as result of genetic transformation and manipulation system for non-*Drosophilar* insects and other insects[20]. The *Mos* 1

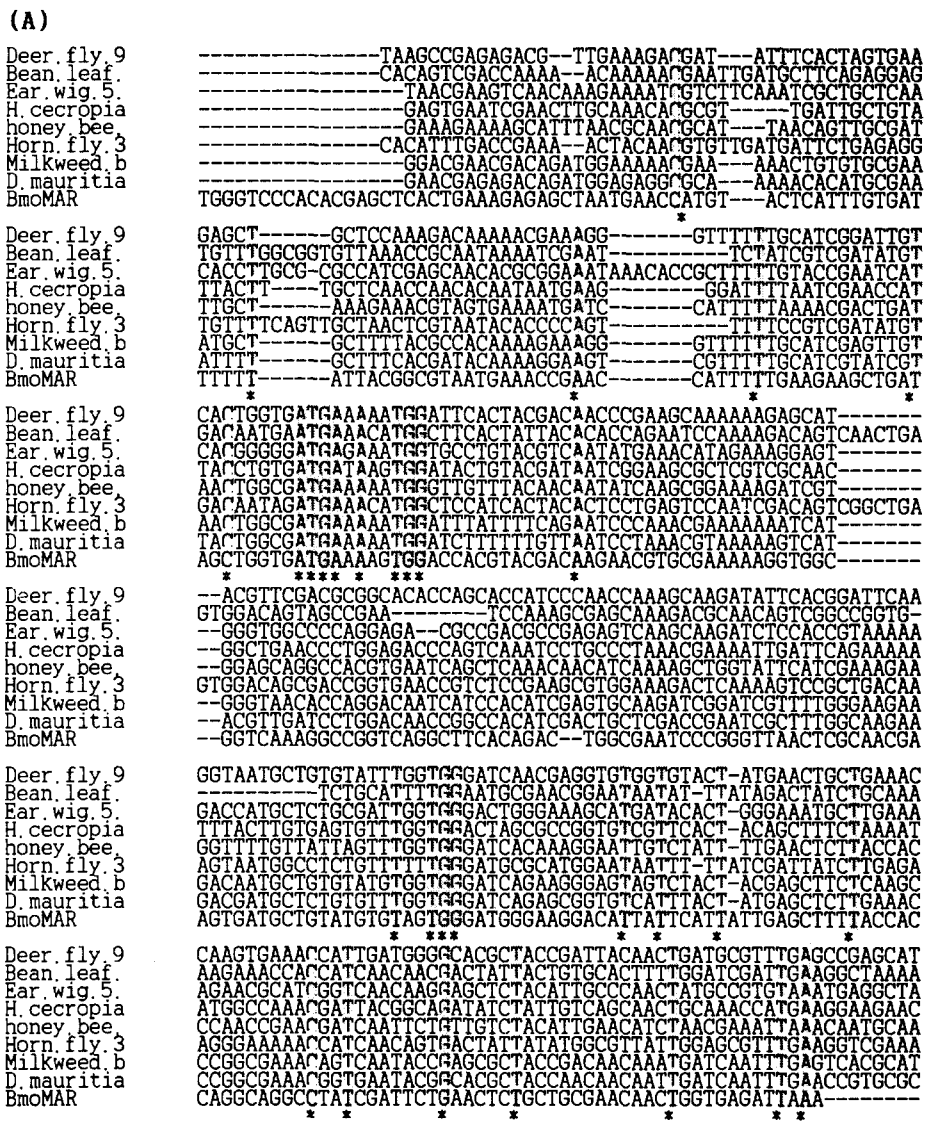

Fig. 4. Alignment of the nucleotide(A) and deduced amino acid(B) sequences of BmoMAR with those of other insects. Origins : Ear. wig. 5 is from *Forticular auricularia,* H. cecropia is from *Hyalophora cecropia,* Horm. fly. 3 is from *Haematobia irritans,* Milkweed. b is *Oncopeltus fasciatus, D. mauritiana* is from *Drosophila mauritiana,* Bean. leaf is from *Cerotoma vittatus,* Deer fly. 9 is from *Chrysops vittatus,* Honey bee is from *Apis mellifera* and BmoMAR is from *Bombyx mori.* Genebank accession numbers for those sequences are L10473, L10443, L10463, L10490, X78906, L10455, L10499, L10433, orderly. In the both sequences, deleted regions are represented by hyphens and asterisks below with gray regions represent the conserved nucleotides and amino acids, respectively.

```
Deer.fly.9      TGCGGGAAAAACGGCCAGAATATGAACAAAGACATGACAAAGTAATTCTTCAACATGACA
Bean.leaf.      TCGCAAAGAAGCGGCCACA-TTTGCGAAAGAAAAAAAT---GTAGTTTCGCCAAAAAAT-
Ear.wig.5.      TGGGACTGAAAAGACCTGA-CC--------GACAAGGCCAAGTCATCTTGCTCCACGACA
H.cecropia      TAGCTGCTAATAAACTGAGATTGGTTAATTGCTCTAGGC---CACTGCTGCTTCACGACA
honey.bee.      TTGAAGAAAAGCGGTTCGAATTGACAAATCGAAAAGGT---GTTGTATTCCATCATGACG
Horn.fly.3      TCGCGGCGAAACGACCCCA-TATGAAAAAGAAAAAAGT---GTCGTTCCACCACGACATC
Milkweed.b      TGATCGAAAAACCGCCAGAATGGGCTCACAGGCACGCCAAAGTCATTCTTCAGCATGATA
D.mauritia      TTCAGAGAAAACGACCGGAATATCAAAAAAGACAACACAAGGTCATTTTTCTCTATGGCA
BmoMAR          ------GCAAGAAGTCGAAATTAATCAACAGAAGGGGT---GTGGTTTTTCATCACGATG
                      **                                  *   *
Deer.fly.9      ACGCTCGGCCACATGTTGCAAATGTCGTGAAGACATACTTGGAAACGCTTCGATGGGATG
Bean.leaf.      GCACCGTGTCACAAATCAATTGAG-------------------TGAATTGGGTTTTGAAT
Ear.wig.5.      ACGCTAGACCCCATGTTGCACAAGTCGTCAAAACCGCACTCCAAGAGCTCGAATGGGAGG
H.cecropia      ACGCAAGACCGTACACTGCACAGCAAACAACCACTAAGTTAGATGAGCTACAATTGGAAT
honey.bee.      ATGCAAGGCCACACACATATTTGGTCACTCGGCAAAAATTATTGGAGCTTGGTTGGGATG
Horn.fly.3      GCACCGTGCCACAAGTCA-TTGAGA-ACGATGGCAAAAATTCATGAATTGGGCTTCGAAT
Milkweed.b      ACGCTCCTGCACATTCAGCCAGATTGACTAAGGAAACGATTTCTTCGCTTGGTTGGGAAC
D.mauritia      ACGCTCCATCACATACGGCAAGAGCGGTTTGCGACACGTTGGAAACACTCAATTGGGAAG
BmoMAR          ACGCTAGACCTCACACATCTTTAGCCACTCAGTAAAAATTATGAGAGCTTGACTGGGATG
                  *     *  *                                 *    *  **
Deer.fly.9      TGCTACCCCACCCGCCA--------------------(Chrysops vittatus; 454bp)
Bean.leaf.      TGCTTACCCCACCCACCC------------------(Cerotoma trifuricata; 426bp)
Ear.wig.5.      TTCTTCAACATCCACCA--------------------(Forficula auricularia; 458bp)
H.cecropia      GTCTGCGACATCCACTG--------------------(Hyalophora cecropia; 450bp)
honey.bee.      TTTTGCCACATTCACTA--------------------(Apis mellifera; 451bp)
Horn.fly.3      TGCTTCCCCACCCACCG--------------------(Haematobia irritans; 463bp)
Milkweed.b      TCCTACCCCACCCGCCG--------------------(Oncopeltus fasciatus; 454bp)
D.mauritia      TGCTTCCGCATGCGGCT--------------------(Drospphila mauritiana; 454bp)
BmoMAR          TGTTAATGCATCCGCCGTATTGACCCGACCTCGCCCC(Bombyx mori; 473bp)
                  *    **  *
```

(B)

```
Ear.wig.5.      ------NEVNKENRLQIAAQHLARHRATRGNKHRFLYRIITGDEKWCLYVNMKHRK---E
H.cecropia      ------SESNLQTRVDCCITLLNQHNNE------GINRTITCDDKWILYDNRKRSS---Q
Horn.fly.3      ------TFDRKLQRVDDSERCFQLLTRNT---PQFFRRYVTIDETWLHHYTPESNRQSAE
Milkweed.b      ------DERQMEKRKTVCEMLLLRHKRK-----GFLHRVVTGDEKWIYFQNPKRKK---S
D.mauritia      ------NERQMERRKNTCEILLSRYKRK-----SFLHRIVTGDEKWIFFVNPKRKK---S
Bean.leaf.      ------TVDQKQKRIDASEECLAVLNRNK---IEFYRRYVTMNETWLHYYTPESKRQSTE
Deer.fly.9      ------KPRDVERRYFTSEELLQRQKRK-----GFLHRIVTGDEKWIHYDNPKQKR---A
honey.bee.      ------KEKHLTQRINSCDLLKKRSEND-----PFLKRLITGDEKWVVYNNIKRKR---S
BmoMAR          WVPHELTERELMNHVLICDFLLRRNETEP-----FLKKLIAGDEKWTTYDKNVRKR----
                        +   +                    + ++  + *
Ear.wig.5.      WVAPGDP-TPRVKQDLHRKKTMLCDWWDWESMIHWEMLEKNASVNKELYIAQLCRVNEAM
H.cecropia      RLNPGDPVKSCPKRKLIQKNLLVSVWWTSAGVVHYSFLKYGQTITADIYCQQLQTMKEEL
Horn.fly.3      WTATGEPSPKRGKTQKSADKVMASVFWDAHGIIFIDYLEKGKTINSDYYMALLERLKVEI
Milkweed.b      WVTPGQSSTSSARSDRFGKKTMLCMWWDQKGVVYYELLKPGETVNTERYRQQMINSHALI
D.mauritia      YVDPGQPATSTARPNRFGKKTMLCVWWDQSGVIYYELLKPGETVNTARYQQQLINLNRAL
Bean.leaf.      WTVA----PKRAKTQQSAG----SAFWNANGIIFIDYLQKETTINNDYYCALLDRLKAKI
Deer.fly.9      YVRRGTPAPSQPKQDIHGFKVMLCIWWDQRGVVYYELLKPSETIDGARYRLQLMRLSRAL
honey.bee.      WSRPRESAQTTSKAGIHRKKVLLLVWWDHKGIVYFELLPPNRTINSVVYIEHLTKLNNAI
BmoMAR          WRSKAGQASQTGESRVNSQR---S--D-AVCVVGWEGHYSLLSFYHQAGLSILNSAANNW
                                          ++          +          +
Ear.wig.5.      GLKRPDRQGQ---VILLHDNARPHVAQVVKTALQELEWEVLQHPP-----(Forficula auricularia; 152aa)
H.cecropia      AANKLRLVNCS-RPLLLHDNARPYTAQQTTTKLDELQLECLRHPL-----(Hyalophora cecropia; 149aa)
Horn.fly.3      AAKRPHMKKK--KVSFHHDIAPCHKSLRTMAKIHELGFELLPHPP-----(Haematobia irritans; 154aa)
Milkweed.b      E-KPPEWAHRHAKVILQHDNAPAHSARLTKETISSLGWELLPHPP-----(Oncopeltus fasciatus; 150aa)
D.mauritia      QRKRPEYQKRQHKVIFLYGNAPSHTARAVCDTLETLNWEVLPHAA-----(Drospphila mauritiana; 151aa)
Bean.leaf.      AKKRPHLRKK--KMVSPKN-APCHKSS--------ELGFELLTHPP-----(Cerotoma trifuricata; 138aa)
Deer.fly.9      REKRPEYEQRHDKVILQHDNARPHVANVVKTYLETLRWDVLPHPP-----(Chrysops vittatus; 151aa)
honey.bee.      EEKRFELTNRK-GVVFHHDDARPHTYLVTRQKLLELGWDVLPHSL-----(Apis mellifera; 150aap)
BmoMAR          DSKKSKLINRR-GVVFHHDDARPHTSLATQK--LELDWDVLMHPPYPDLA(Bombyx mori; 152aa)
                                  *                 *  + * *
```

Fig. 4. (Continued)

element of *D, mauritiana* can transposite into the genome of *D, melanogaster.* It is possible that it act functionally in genome of all insects, and then the element from close relatives might be used for the target organisms. In future study, at least one clone derived from *B. mori's* mariner-like transposon including a clone isolated in this study will be characterized/recovered and analysed its full-length BmoMAR and determined its copy number in the genome, and then the element will be a strong useful tools for construction of transgenic silkworms to study and to breeding under molecular level.

References

1. Berg, D. E. & Howe, M. M : Mobile DNA. American Society of Microbiology(1989).
2. Finnegan, D. J. : Transposable element and DNA transposition in eukaryotes. *Curr. Biol.* 2, 471-477 (1990).
3. Finnegan, D. J. & Fawcett, D. H. : Transposable elements in *Drosophila melanogaster.* Oxford Survey of Eukaryotic Genes, 3, 1-62(1986).
4. Medhora, M., Maruyama, K. & Hartl. D. L. : Molecular and functional analysis of mariner mutator element Mos1 in *Drosophila. Genetics* 128, 311-318 (1991).
5. Mullins, M. C., Rio, D. C. & Rubin, G. M. : Cis-acting DNA sequence requirements for P-element transposition. *Genes & Develp.,* 3, 729-738(1989).
6. Jacobson, J. L., Medhora, M. M. & Hartl, D. L. : Molecular structure of a somatically unstable transposable element in Drosophila. *Proc. Natl. Acad. Sci. USA.* 83, 8684-8688(1986).
7. Garcia, F., Marfany, G., Bagina, J. & Salo, E. : Infiltration of mariner elements. *Nature* 363, 109(1993).
8. Robertson, H. M. & MacLeod, E. G. : A mariner transposable element from a lacewing. *Nucl. Acid Res.* 20, 6409(1992).
9. Robertson, H. M. : The mariner transposable element is widespread in insects. *Nature* 362, 241-245 (1993).
10. Iatrou, K. & Meidinger, R. G. : Tissue-specific expression of silkmoth chorion genes in vivo using Bombyx mori nuclear polyhedrosis virus as a transducing vector. *Proc. Natl. Acad. Sci. USA.* 87, 3650-3654(1990).
11. Tamura, T., Kanda, T., Takiya, S., Okano, K. & Maekawa, H. : Transient expression of chimeric CAT genes injected into early embryos of the domesticated silkworm *Bombyx mori. Jpn. J. Genet.* 65, 401-410(1990).
12. Jasinskiene, N., Coates, C. J., Benedict, M. Q., Cornel, A. J., Rafferty, C. S., James, A. A. & Collins, F. H. : Stable transformation of the yellow fever mosquuito with the *Hermes* element from the housefly. *Proc. Natl. Acad. Sci. USA.* 95, 3743-3747(1998).
13. Coates, C. J., Jasinskiene, N., Miyashiro, L. & James, A. A. : Mariner transposition and transformation of the yellow fever mosquito. *Proc. Natl. Acad. Sci. USA.* 95, 3748-3751(1998).
14. Thompson, I. D., Higgins, D. F. & Gibson, T. J. : CLUSTAL W ; improving the sensitivity of progressive multiple sequence alignment through sequence weighing, position-specific gap penalties and weight matrix choice. Nucl. Acid Res. 22, 4673-4680(1994).
15. Bigot, Y., Hamelin, M. H., Capy, P. & Periquit, G. : Mariner-like elements in hymenopteran species : insertion site and distribution. *Proc. Natl. Acad. Sci. USA.* 91, 3408-3412(1994).
16. Elbert, P. R., Hilemen, J. P. IV. & Nguyen, H. T. : Primary sequence, copy number, and distribution of mariner transposon in honey bee. *Insect Mol. Biol.* 4, 69-78(1995).
17. Sendensky, M. M., Hudson, S. J., Everson B. & Morgan, P. G. : Identification of mariner-like repetitive sequence in C. elegans. *Nucl. Acid Res.* 22, 1719-1723(1994).
18. Lidholm, D. A., Gudmunderson, H. G. & Boman, H. G. : A highly repetitive, mariner-like element in the genome of *Hyalophora cecropia. J. Biol. Chem.,* 266, 11518-11521(1991).
19. Anaysis of eukaryotic mRNA structures dircting co-translational incorporation of selenocysteine. *Nucl. Acid Res.* 24, 1195-1201(1994).
20, Blanchetot, A & Gooding, R. H. : Identification of a mariner element from the tsete fly *Glossina palpalis palpalis. Insect Mol. Biol.* 4, 89-96(1995).

초록 : 누에에서의 Mariner 유사 전이인자유전자의 동정

이진성 · 황재삼* · 김용성 · 서동상** · 권오유†***
(생명공학연구소 게놈사업단, *농촌진흥청 잠사곤충연구소, **성균관대학교 유전공학과, ***충남대학교 해부학교실)

이미 밝혀져 있는 mariner 전이인자의 전이효소를 암호화하는 부위에 대하여 퇴화성 primer를 사용하여 PCR 방법에 의해서 누에(*Bombyx mori*)에서 mariner 유사 전이인자의 잠정적인 전이효소 부위를 클로닝하였다. BmoMAR로 명명된 이 PCR 클론으로부터 추론된 아미노산은 152개로 다섯 개의 종결코돈이 삽입되어 있었으며, *Drosophila mauritiana*의 active Mos 1에 37%의 아미노산 상동성을 보였다. 또한, 기존의 곤충들에서 밝혀진 mariner-like element에 대한 상동성은 DNA 수준에서는 *Apis mellifera*에 59% 그리고 아미노산 수준에서는 *D. mauritiana* 7.9 clone에 37% 상동성을 보였다. 이 결과는 mariner-like element가 *B. mori*에도 존재하고있지만, 이들 전이인자의 전이효소를 암호화하는 부위에 종결코돈이 발견되는 것으로 보아서 비활성 전이인자 혹은 일종의 selenoprotein으로 추정된다.