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Identification and chromosomal localization of *mariner*-like elements in the cabbage moth *Mamestra brassicae* (Lepidoptera)

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Abstract

A complete *mariner*-like element has been identified in the lepidopteran *Mamestra brassicae*. This element, called *Mbmar*, represents a new type of *mariner* transposon. It has a transposase similar to that of other insect *mariner* coding sequences but its inverted terminal repeats differ from typical *mariner* ones. This observation is unique since generally both *mariner* coding region and ITRs are evolutionarily conserved in insects. *Mbmar* is detectable by FISH only in the heterochromatic regions of both the sex chromosomes.

Introduction

A significant portion of the prokaryotic and eukaryotic genome consists of interspersed and moderately repeated sequences that are able to transpose in the host genome. In eukaryotes, transposable elements are divided into two main classes according to their structural organization and mechanism of transposition (Pimpinelli *et al.* 1995). Class I elements use an RNA-mediated mechanism of transposition and they encode for a reverse transcriptase. Class II mobile DNAs (which are transposons *in sensu strictu*) use a DNA-based mode of transposition (Pimpinelli *et al.* 1995).

The most widespread mobile DNA is *mariner* since it is present from fungal genomes to invertebrates and vertebrates (Robertson 1993, Capy *et al.* 1994, Auge-Goiullou *et al.* 1995, Mandrioli 2000). *Mariner*-like elements (MLEs) are class II transposons that are about 1.3 kilobase pairs (kbp) long. They encode for a single protein, called transposase, that is involved in MLE mobilization. The transposase-coding region is located in the central portion of the transposon and is generally flanked by 20–30 bp inverted terminal repeats (ITRs) (Robertson 1993, Capy *et al.* 1994, Auge-Goiullou *et al.* 1995).

In this paper, the identification and chromosomal localization of a new type of *mariner*-like element is reported in the genome of the cabbage moth *Mamestra brassicae*.

Materials and methods

Genomic DNAs were extracted from *Mamestra brassicae* CRL-8003 cells (ATCC, USA) as reported in Mandrioli (2002).

The presence of MLEs in the *M. brassicae* genome was evaluated by PCR using the primers F_{MAR} (5′-GGAAGTCGTTTTTGCYTCNT) and R_{MAR} (5′-ATCGANCCATTCTTCCZCWC),

corresponding to the central portion of *mariner* transposase. The amplification mix contained 100 ng of genomic DNA, 1μmol/L of each primer, 200 μmol/L dNTPs and 2 U of DyNAZyme II polymerase (Finnzymes Oy). Amplification was performed with a Hybaid thermalcycler at an annealing temperature of 59°C for 1 min and making extension at 72°C for 1 min.

MLE sequence was completed by inverse PCR with the primers $F_{MAR\text{-}I}$ (5′-CCTAGTCTCGCCACAVTAAW) and $R_{MAR\text{-}I}$ (5′-CGGACCAGACCACDCATTBC) according to Martin & Mohn (1999). A complete *mariner* element was finally amplified using the primers F_{MAR2} (5′-TGACAGTTGCATCGTAGCTGC) and R_{MAR2} (5′-AGCTTAAACGCACGGCGGCATA), designed on the basis of *mariner* flanking sequences previously isolated by inverse PCR.

All the amplified fragments were cloned using the pGEM T-easy cloning kit (Promega). Sequencing was performed at the "BMR-University of Padova". Sequence analysis was made using GCG Software.

Southern blotting was realized according to Mandrioli *et al.* (1999a) making stringency washes at 68°C in 0.1 × SSC twice for 15 min. Chromosome preparation and fluorescent *in-situ* hybridization (FISH) were made as previously described (Mandrioli *et al.* 1999b).

Results

PCR amplification, performed with primers designed on the central portion of *mariner* transposase, showed a 500-bp-long fragment (Figure 1a). The *M. brassicae mariner* sequence has been completed by inverse PCR that showed 6 bands ranging from 1100 to 2500 bp (Figure 1a).

A complete *mariner*-like element has been obtained from the *M. brassicae* genome using primers designed on the previously isolated MLE flanking sequences. The sequence of this element can be found in GeneBank, Accession Number AF465247.

The *M. brassicae mariner*-like element (called *Mbmar*) shows a sequence similarity ranging from 91.4% to 94% with *mariner* elements isolated from *Musca domestica* (AF373028) and *Drosophila mauritiana* (DMMELE1), respectively. In particular, alignment indicated that the *Mbmar* transposase coding sequence is highly conserved (97%) whereas the inverted terminal repeat (ITR) sequences are completely different in *M. brassicae mariner* with respect to other *mariner* ITRs. This kind of observation is unique for *mariners* since generally both coding region and ITRs were evolutionary conserved.

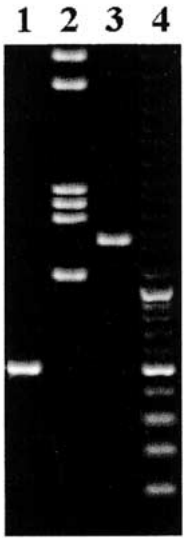

Figure 1. A central portion of *mariner* transposase was amplified from *M. brassicae* genome by PCR (lane 1). Amplification of *mariner* flanking sequences was performed by inverse PCR and evidenced 6 bands ranging from 1100 to 2500 bp (lane 2). The sequence of a *mariner* flanking region was used as target for new primers that allowed us to amplify the complete 1291 bp MLE in the cabbage moth (lane 3). The molecular weight of the amplified fragments has been evaluated using a 100-bp ladder marker (lane 4).

Amplification of *mariner* flanking sequences indicated that *Mbmar* is inserted into a TA target sequence that was duplicated after transposon insertion (Figure 2).

Southern blotting with the complete *Mbmar* as a probe displayed large bands corresponding to internal fragments of the *Mbmar* sequence and a diffuse smear at high molecular weight. This is probably constituted by *Mbmar* elements interspersed into the cabbage moth genome (Figure 3).

FISH experiments with the *Mbmar* probe showed large fluorescent regions limited to the two sex chromosomes. In particular, MLE were clustered in heterochromatic regions of Z and W chromosomes whereas no autosomal cluster could be observed even if FISH experiments were

Figure 2. Alignment of *Mbmar* flanking sequences indicated that the *Mbmar* is inserted into the TA target sequence that is duplicated after transposon insertion. *Mbmar* termini are boxed. Asterisks indicate conserved nucleotides.

performed at lower stringency (Figure 4). However, FISH cannot exclude the presence of single interspersed *Mbmar* copies since these sequences could escape detection by FISH.

Discussion

The *mariner* transposon family is peculiar among class II transposable elements in view of its extraordinary distribution (Robertson 1993, Capy *et al.* 1994, Auge-Gouillou *et al.* 1995). It is therefore not surprising to find an MLE in the genome of the cabbage moth. However, analysis of *Mbmar* sequence showed that its structure was peculiar. *Mbmar* transposase is highly similar to other MLE transposases, whereas the ITR structure is unique and has completely diverged from that previously described in other *mariners*.

Southern blotting with the *Mbmar* probe showed several hybridization signals indicating that numerous *Mbmar* elements were present in *M. brassicae* genome. Transposable elements are generally not randomly distributed on chromosomes (Boyle *et al.* 1990, Pimpinelli *et al.* 1995, Dimitri & Junakovic 1999). FISH showed that *Mbmar* clusters were located in heterochromatic regions of *M. brassicae* Z and W chromosomes. This result could be partially explained considering that, in *M. brassicae*, a large proportion of the heterochromatin is on the sex chromosomes (Mandrioli *et al.* 2003), so that the transposons could be said to be located in the wider heterochromatic district of the complement. However, it is also possible that the sex chromosomes have acted as a trap for transposable elements, as reported in *Drosophila melanogaster* (Steinemann & Steinemann 1992).

The fact that the same mobile DNA was located in both sex chromosomes but absent in all autosomes is of a particular interest. Taken together with the absence of recombination in lepidopteran females (for a review, see Marec 1996), it

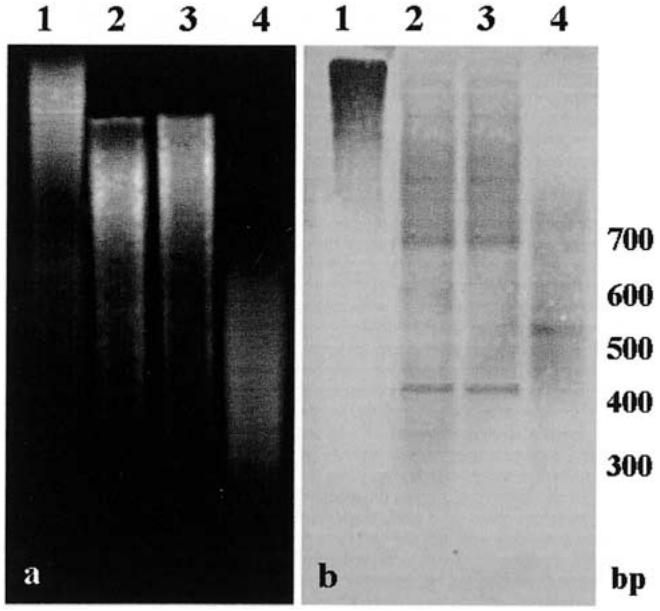

Figure 3. (**a**) *M. brassicae* genomic DNA digested with *Eco*RI (lane 1), *Msp*I (lane 2), *Hpa*II (lane 3) and *Sau*3A (lane 4) and hybridized with the *Mbmar* probe (**b**).

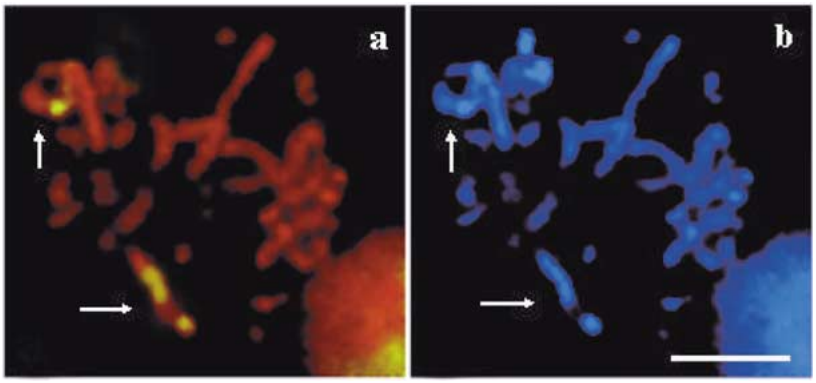

Figure 4. (**a**) FISH shows two large *Mbmar* clusters located in heterochromatic regions of Z and W chromosomes, as deduced by C banding and DAPI staining, of the same mitotic plates (**b**). Arrows indicate Z and W chromosomes. Bar corresponds to 10 μm.

could imply that mobile elements were already present in these chromosomes before they had differentiated as sex chromosomes or that they could be involved in the differentiation of sex chromosomes.

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