Cah. Biol. Mar. (2002) 43 : 325-328

Mariner transposons are widespread genetic parasites in the genome of hydrothermal invertebrates

Nathalie CASSE[1,3], Elisabeth PRADIER[2], Marie-Véronique DEMATTEI[2], Yves BIGOT[2] and Marc LAULIER[1]
(1) *Laboratoire de Biologie et de Génétique Évolutive, Université du Maine, Avenue O. Messiaen 72085 Le Mans, France.*
(2) *Laboratoire d'Etude des Parasites Génétiques,*
Université François Rabelais, Avenue Monge 37200 Tours, France.
(3) *Corresponding author : Nathalie Casse, Laboratoire de Biologie et de Génétique Évolutive,*
Université du Maine, Avenue O. Messiaen 72085 Le Mans, France
Fax (33) 02 43 83 39 17 - E-mail : Nathalie.Casse@univ-lemans.fr

Introduction

A transposon is a mobile DNA sequence, a so-called transposable or mobile element. It can move to different positions in the genome, and the mechanism that permits this mobility is known as transposition. Transposons are currently considered to be molecular parasites, because their mobility may cause mutations and they have a strong invasive capacity. However, their ability to provide a source of genetic variability for the genome of their host means that they can also be viewed as beneficial genetic factors for their host genome (Kidwell & Lisch, 2001). Transposons integrated into their host genome are transmitted from parent to offspring (vertical transfer). Moreover, they are also transmitted between species during evolution, by a process known as horizontal transfer. Numerous studies have shown that the transposition mechanism can be use to design vectors for transfer of genes in eucaryotic species.

The mariner transposons belong to a family related elements widespread in eucaryotic genomes known as mariner-like elements (MLEs; for review, see Plasterk et al., 1999). These transposons are about 1300 base pairs in length, and they contain a single gene that encodes for the transposase. At their 5' and 3' extremities, MLEs are flanked by inverted terminal repeats (ITRs) of about 28-30 pb (Fig. 1). Mariner transposition is a "cut and paste" mechanism that allows the transposon to excise itself from the original site to insert itself into a new target site located elsewhere in the host genome. The ability to transpose requires just one enzyme – a transposase – and this is the main, and frequently the only, protein encoded by the transposon.

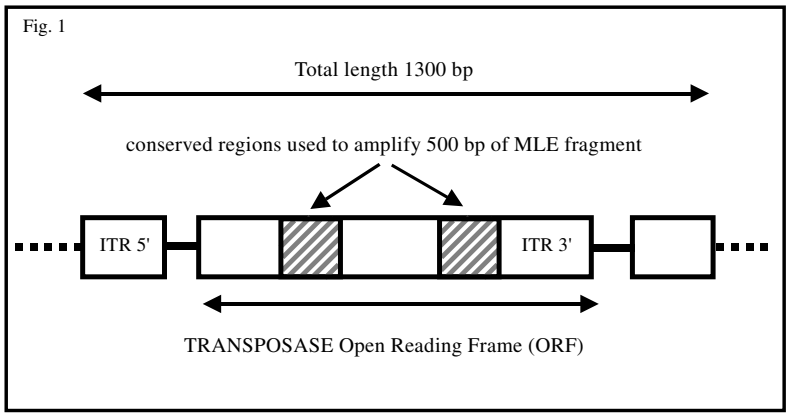
The severe environmental conditions to which they are subjected and the isolation of the hydrothermal invertebrates makes them interesting biological models with which to study the mechanisms and elements able to create genetic variability, such as transposons. The present study describes MLEs in several hydrothermal species : *Bythograea thermydron* (Williams, 1980), *Rimicaris exoculata* (Williams & Hesseler, 1986), *Bathymodiolus thermophilus* (Kenk & Wilson, 1985), and *Riftia pachyptila* (Jones, 1981). Due to the presence of stop codons and frame shifts, the MLE sequences obtained from hydrothermal organisms seem to be unable to encode an active transposase, with the exception of one isolated from *B. thermydron*. The ability of this MLE to trigger transposition will be confirmed using a bacterial assay.

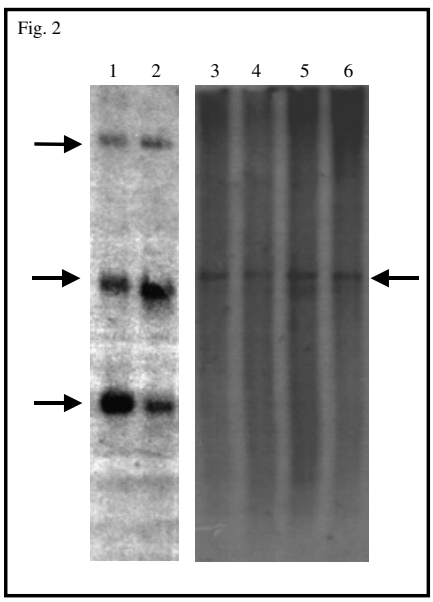
Material and methods

The hydrothermal specimens tested were collected during missions HERO 94, MARVEL II 97 (Açores), and HOPE 99 missions. Specimens were frozen in liquid nitrogen for conservation. The hydrothermal species studied are : *Bythograea thermydron, Rimicaris exoculata* (Arthropoda), *Bathymodiolus thermophilus* (Mollusca), and *Riftia pachyptila* (Annelida).

Genomic DNAs were purified described by Laulier et al. (1995). An internal fragment of MLE transposons was first amplified by Polymerase Chain Reaction (PCR) using two internal conserved regions in the MLE transposase sequence as primers (MAR 124F and MAR 276R, Roberston, 1993). This made it possible to clone fragments about 500 bp long in PGEM-T Easy plasmid (Promega), and then to sequence them. These fragments were also used as radiolabeled probes in Southern blot/hybridization experiments.

The ITRs were determined from sequences obtained by Inverted Polymerase Chain Reaction (IPCR). The primers





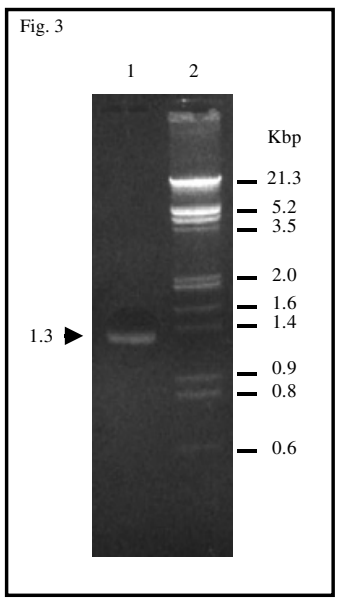


Figure 1 : Structure of the *mariner* transposon. Its total length is approximately 1300bp. The ITRs are small DNA sequences (about 30 bp long) with an inverted orientation .The *Mariner* element encodes a single gene, the transposase. The two dashed boxes indicate the amino acid conserved motifs WVPHEL and YSPDLAP, used to design the primers for the PCR amplifications. Dashed lines indicate the chromosomal DNA.

Figure 2. Estimation of the mariner transposon copy number (arrows) in the genome of two individuals of *R. exoculata* (lanes 1-2) and four of *R. pachyptila* (lanes 3-6) by Southern blot and hybridization with the 500 pb MLE fragments cloned from *B. thermydron* (lanes 1-2) or *R. pachyptila* (lanes 3-6). Genomic DNAs were digested by *Eco*RI, a restriction enzyme that is expected to cut outside the MLEs present in these species.

Figure 3. Detection of a 1300 bp (arrow head) DNA fragment amplified by PCR using the *B. thermydron* genome as DNA source, and primers designed from the ITR sequences defined from IPCR results in *B. thermydron***.** Amplification products (lane 1) were separated by electrophoresis (1% gel agarose), stained with ethidium bromide and photographed under UV light. The DNA molecular weight HindIII/EcoRI is shown in lane 2 and band sizes are indicated in the margin.

and the restriction enzymes used for the IPCR to allow the cloning of 5' and 3' ITR were designed on the basis of the sequence of 500 pb MLE fragment and following a procedure described elsewhere (Ausubel et al., 1994). The ITR sequences were used to design PCR primers to amplify the full-length MLE approximately 1300 bp long, and these were then cloned and sequenced.

Results

Fragments of 500 bp were successfully amplified by PCR from *B. thermydron*, *B. thermophilus* and *R. Pachyptila* (Acc. N° AJ276071, AJ276072 and AJ276073). A comparison of the nucleic acid sequences obtained from these species showed that the *B. thermydron* and *B. thermophilus* fragments were 97% similar sequences and that those found in *R. pachyptila* 51 and 48% similar to those of *B. thermydron* and *B. thermophilus,* respectively.

The Southern blot/hybridization analyses revealed at least three copies of MLE in the genome of *R. exoculata* (Fig. 2, lanes 1 & 2), whereas only one copy was detected in the genome of *R. pachyptila* (Fig. 2, lanes 3 - 6). Using PCR and IPCR experiments (Fig. 3), made it possible to clone and sequence a full-length MLE in *B. thermydron* genome that was named Bytmar1. This MLE is 1298 bp long, its 5' and 3' ITRs are respectively 36 and 37 bp long (Fig. 4). Its nucleic acid sequence contains an intact open reading frame encoding a transposase of 350 amino acids.

Discussion

The estimation of the copy number of MLEs in the genome of *R. exoculata* and *R. pachyptila* is low, but is within the range reported for other terrestrial species ranging from 2 to 3 copies per genome in the nematode *Meloidogyne spp*. or in *Drosophila melanogaster*, to 8,000 copies in the planarian *Dugesia tigrina* and 15,000 in the leptidoptera *Hyalophora cecropia* (for review, see Plasterk et al., 1999).

Since the mariner element has been discovered in *Drosophila mauritiana* (insect) by Jacobson & Hartl (1985), many other MLEs have been reported and these elements are now considered as ubiquitous in eukaryotic species. In the literature, numerous authors are interested in the mobility of the MLEs in the eucaryotic genomes, and most of MLEs described correspond to elements that have lost their ability to encode an active transposase due to the presence of frame shifts and stop codon in the ORF. So far, Mos-1 is the only naturally active mariner transposon to have been isolated from *D. mauritiana* (Medhora *et al*., 1991). In this study, we described a full-length MLE, Bytmar1, that contains an ORF, and that may correspond to a new putative naturally-

```
1    TACGAGGGGCGGTCAGAAAGTTATGCAATTCGGTATGTTAGCTAGCTCAACCAGTCGTGA
     CCTGTTGTGTCCGGCTAGGACTGGCTCTGACATGTTCACAAACAGCGGCCTGCGCTCCCT
121  CGCTCCCTCTTTAGTCTTAGGGGAGCGAACCAGTGGACGCGGTTTGCAGGAATCGGCATC
     AATGGGCAAGATCGAGTACCATGCAGTGATCAAGTTCTTGACAAAAGTGGGGAAGAACGC
       M  G  K  I  E  Y  H  A  V  I  K  F  L  T  K  V  G  K  N  A  20
241  GAAGGAGATCCACGACAGGCTGGTTGCGGTGTACAACGACACTGCCTCTTCGTATGCCAC
       K  E  I  H  D  R  L  V  A  V  Y  N  D  T  A  S  S  Y  A  T  40
     AGTCACCCGCTGGCACAAGGAATTTCGTCATGGCCGTGAGTCCCTTGAAGACGACTCCCG
       V  T  R  W  H  K  E  F  R  H  G  R  E  S  L  E  D  D  S  R  60
361  TGTGGGACGCACCTTCGAGGCGACTTCCGAAGACACTGTTGACCGTGTGGAGGCAATGAT
       V  G  R  T  F  E  A  T  S  E  D  T  V  D  R  V  E  A  M  I  80
     CATGGAAAATCGGCGAGTGAAGGTGGAGGAAATTTCGTTGGAGATTAGAATTTCTCATGG
       M  E  N  R  R  V  K  V  E  E  I  S  L  E  I  R  I  S  H  G  100
481  AAGCGTTTGCACCATTATTAATCATCACCTGGGCATGAGCAAAGTTTCTGCTCGTTGGGT
       S  V  C  T  I  I  N  H  H  L  G  M  S  K  V  S  A  R  W  V  120
     GCCCCGAAATCTTTCTCTGCATGATCGCCTTCAGGGCCAAACAAGTTCGGAGGAGCTGCT
       P  R  N  L  S  L  H  D  R  L  Q  G  Q  T  S  S  E  E  L  L  140
601  GACTCTGTACAACGCATACCCAGCGGGATTCAAGTCGAGGGTCATGACAGGTGATGAAAC
       T  L  Y  N  A  Y  P  A  G  F  K  S  R  V  M  T  G  D  E  T  160
     GTGGGTTCACCACTGGGACCCAGAGACGAAGCTCGAGAGTATGGCCTGGAAACAGAAGGG
       W  V  H  H  W  D  P  E  T  K  L  E  S  M  A  W  K  Q  K  G  180
721  ATCGCCGACACCGCTCAAGTTTTGGACCCAACCATTGGCTGGCAAGATCATGGCCACCAT
       S  P  T  P  L  K  F  W  T  Q  P  L  A  G  K  I  M  A  T  I  200
     CTTCTGGGACGCCGGGGGGGTGCTGCTGGTGGACGTCCTGCCGCGTGGCTCTACGATCAC
       F  W  D  A  G  G  V  L  L  V  D  V  L  P  R  G  S  T  I  T  220
841  GGGGAAGTACTACGCCGGAGTACTCGGTCGCTTGAGGGACTCCATCCGTCAGAAGAGGCG
       G  K  Y  Y  A  G  V  L  G  R  L  R  D  S  I  R  Q  K  R  R  240
     GGGCAAGTTGACCCGTGGTGTCCTCCTCCTCCTCCATGACAACGCTCCGGTCCACAAGGC
       G  K  L  T  R  G  V  L  L  L  L  H  D  N  A  P  V  H  K  A  260
961  CCACCATGCCCAGGCTGCTCTGAGGGACTGTGGCTTCGAGCAGTTCAATCACCCATCCTA
       H  H  A  Q  A  A  L  R  D  C  G  F  E  Q  F  N  H  P  S  Y  280
     CAGTCCGGACCTGGCCCCCAATGACTACTTTCTGTTCCGCCAGCTCAAGTCCTCGTTGCG
       S  P  D  L  A  P  N  D  Y  F  L  F  R  Q  L  K  S  S  L  R  300
1081 GGGGCGGAGGTTTGACGACAATGATGAGGTCAAGGAGGCTGTGATGATGTGGTTGGAGGA
       G  R  R  F  D  D  N  D  E  V  K  E  A  V  M  M  W  L  E  E  320
     GCAGTTGGAATCCTTCTGGCTGGCAGGAATCCAGAGCCCTTCGCGACAAGTGGTTCAAAT
       Q  L  E  S  F  W  L  A  G  I  Q  S  P  S  R  Q  V  V  Q  M  340
1201 GTATTCAATCAAAGGTAATTACATTGAAAAATGATGTGGTTATCACTTTCATTCCTCCGA
       Y  S  I  K  G  N  Y  I  E  K  .                              360
1261 AATAAATACCGGAATTGCAGAACTTTCTGACCTCCCCTTGTA
```

Figure 4. Nucleotide sequence and amino acid translation of Bytmar 1. The inverted terminal repeats are bold underlined.

occuring element encoding for an active transposase. The ability of Bytmar 1 to perform some or all the transposition steps was checked by bacterial assay (Augé-Gouillou et al., 2001).

Acknowledgements

We would like to thank the Senior Scientists, D. Desbruyères and F. Lallier, the captains and crews of the MARVEL 97and HOPE 99 missions for their support.

We also thank Richard Cosson (EP 61 CNRS-Isomer, Nantes, France) for providing some of the deep-sea specimens of the HERO 94 mission.

References

Augé-Gouillou C., Hamelin M.H., Demattei M.V., Periquet G. & Bigot Y. 2001. The wild type conformation of the Mos-1 inverted terminal repeats is not the most efficient solution for transposition in bacteria. *Molecular Genome and Genetics*, **265:** 51-57.

Ausubel F.M., Brent R., Kingston R.E., Morre D.D., Seidman J.G., Smith J. A. & Struhl K. 1994. *Current protocols in molecular biology*. **1** & **2** pp. Jhon Wiley and Sons, Inc.

Jacobson J.W & Hartl D.L. 1985. Coupled instability of two X-linked genes in *Drosophila mauritiana* : germinal and somatic mutability. *Genetics*, **111**: 57-65.

Kidwell M.G. & Lisch D. 2001. Perspective : transposable elements, parasitic DNA and genome evolution. *Evolution*, **55**: 1-24.

Laulier M., Pradier E., Bigot Y. & Périquet G. 1995. An easy method for preserving nucleic acids in field samples for later molecular and genetic studies without refrigerating. *Journal of Evolutionary Biology*, **8**: 657-663.

Medhora M., Maruyama K. & Hartl D.L 1991. Molecular and functional analysis of the mariner mutator element Mos1 in *Drosophila*. *Genetics*, **128**: 311-318.

Plasterk R.H.A, Izsvak Z. & Ivics Z. 1999. Resident aliens, the Tc1/mariner superfamily of transposable elements. *Trends in Genetics*, **15**: 326-332.

Robertson H.M. 1993. The mariner transposable element is widespread in insects. *Nature*, **362**: 241-245.