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Short communication

Transposition of the *Drosophila* element *mariner* within the human malaria parasite *Plasmodium falciparum*

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Plasmodium falciparum is an important intraerythrocytic protozoan pathogen, responsible for the most severe form of human malaria. Tools that allow large scale functional analysis of this genome are needed to exploit the accumulating *P. falciparum* genomic information, for better understanding of the biology of the parasite and to guide drug and vaccine development. While DNA transfection of this organism has been accomplished, the methods are still improving and recombination is nearly always at homologous sites [1]. The establishment of a transposable element system for *Plasmodium* would allow rapid generation of gene fusions and insertional mutants. Towards this goal, we demonstrate transposition of the autonomous *Drosophila mauritania* element *mariner* within *P. falciparum*.

Transposons of the *mariner/Tc1* family are ubiquitous elements of eukaryotic genomes capable of moving in a 'cut and paste' manner independent of host-specific factors [2–4]. They include inverted terminal repeat (IR) sequences (28-base pair) that are recognized by a transposase, the only gene encoded by the transposon, allowing the excision of the transposon and its insertion into a TA dinucleotide anywhere in the genome. Notably, host factors are not essential for transposition, allowing function of transposable elements in a variety of prokaryotes and eukaryotes [5,6].

Plasmid pMD (Fig. 1A) contains the autonomously active MOS1 transposon, inserted into the shuttle vector pHD22Y which contains a selectable human DHFR marker [7]. pMD was introduced into *P. falciparum* clone 3D7 by electroporation and transfectants were obtained following 4 weeks of culture in the presence of methotrexate, as described [7]. Transfectant pools obtained in this manner contain multi-copy episomal DNA, as revealed by Southern blot analysis for the pMD transfectants (Fig. 1B). Additionally, the pMD transfectants showed the presence of

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fainter bands suggestive of transposition, as seen previously in *Leishmania* [5]. The products of putative transposition events were recovered from these new fragments by an inverted PCR approach, and the DNA sequence determined. Many of the fragments had episomal sequences or internal (non IR) transposon sequences followed by *Plasmodium* DNA, likely resulting from recombination events after recovery in *E. coli* or PCR artifacts (although non-canonical transposase-mediated rearrangements cannot be ruled out). Significantly, five fragments showed evidence of bona-fide transposition, with replacement of *Drosophila* sequences from the transposon donor plasmid pMD by *P. falciparum* sequences. Consistent with the TA target site preference for *mariner*

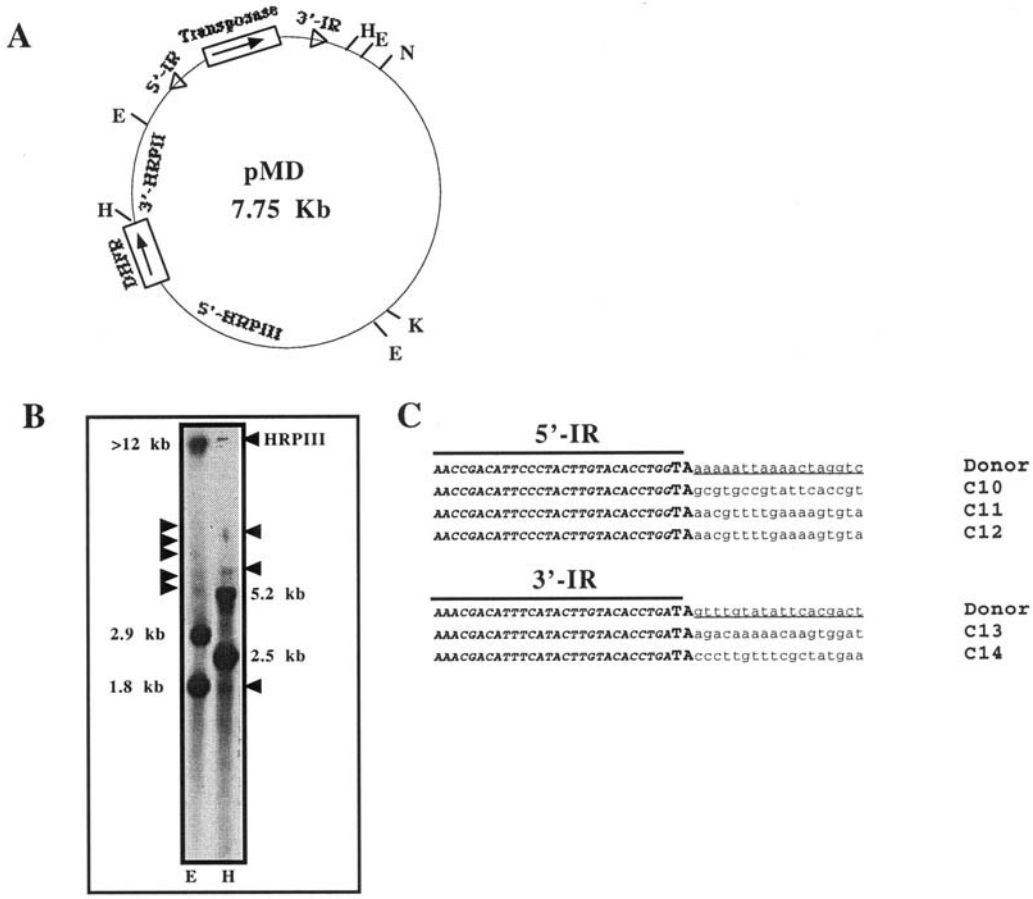

Fig. 1. (A) Diagram of the *mariner* transposon donor plasmid (pMD). E: *Eco*RI, H: *Hind*III, K: *Kpn*I, N: *Not*I. The triangles represent inverted repeats (IR) that delimit the ends of the MOS1 element (B) Southern analysis: genomic DNA from drug resistant parasites was digested with *Eco*RI and *Hind*III and probed with a 4.9 kb *Kpn*I-*Not*I fragment from the pMD vector carrying the 5′-HRPIII, human DHFR marker, 3′-HRPII and the MOS1 element. (C) Inverse PCR: genomic DNAs were separated on agarose gels after restriction digestion as in (B), and DNA fragments in the size range for the new mariner-hybridizing band (arrows in part B) were isolated. These were then digested with *Sau*3A, self-ligated, and used in standard PCR reactions. The 5′ primers (as defined by the transposase ORF within the Mos1 element) were (5-GCCGAACTGCAAGCATTATTGG; sense) and (5-TGAAGCGTTGAAACCACCGTTC; antisense); the 3′ primers were (5-TCCACAAATTGCCCGAGAGATG; sense) and (5-ATGTGATGGAGCGTTGTCATGG; antisense). Products differing in size from those arising from the parental plasmids were identified by gel electrophoresis, inserted into pCRII vector (Invitrogen), and sequenced. The *D. mauritiana* inverted repeat (IR) sequences present in the Mos1 donor plasmid are in bold and italics and the TA dinucleotides marking the boundary between mariner and *Plasmodium* sequences are in bold and large type. The *Drosophila* donor sequences are underlined.

elements, this rearrangement occurred at the expected TA dinucleotide (Fig. 1C). While these experiments do not allow an accurate estimate of the transposition frequency, their presence in the uncloned, unselected primary pMD transfectant population is encouraging.

Interestingly, three of the insertions were in the coding region of the protein kinase A gene. Two are the same and probably are not of independent origin (Fig. 1C, C11 and C12). One has an insertion 25 nucleotides upstream and in the opposite orientation (Fig. 1C, C13). Southern blot analysis of an isolated clone confirms integration into the protein kinase A locus (not shown). Whether insertion at this gene is favored or confers a growth advantage has not been established.

At this time we do not know the source of transposase responsible for mediating the observed transposition. Potentially, expression of transposase from the MOS1 element itself occurs in *P. falciparum*. However, western blots and immunofluorescence as well as RT-PCR experiments failed to detect transposase protein or RNA expression in the parasites (data not shown). Alternatively, transposase may be provided in trans by endogenous *mariner* family elements within the *P. falciparum* genome. *Mariner* elements are widespread in evolution, occurring in all kingdoms of life [8].

These studies establish the occurrence of *mariner* transposition within the *P. falciparum* genome. Future efforts will focus on generation of transposon insertion libraries. Such genome-wide insertional mutagenesis should lead to significant advances in understanding the biology of this important pathogen.

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