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Mutant *Mos1 mariner* transposons are hyperactive in *Aedes aegypti*

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Abstract

The development of genetic strategies to control the spread of mosquito-borne diseases through the use of class II transposons has been hampered by suboptimal rates of transformation and the absence of post-integration mobility for all transposons evaluated to date. Two *Mos1 mariner* transposase mutants were produced by the site-directed mutagenesis of amino acids, E137 and E264, to K and R, respectively. The effects of these mutations on the transpositional activities of *Mos1*-derived transposon constructs were evaluated by interplasmid transposition assays in *Escherichia coli* and *Aedes aegypti*. The transpositional activities of two *Mos1* transposons, one with imperfect wild type inverted terminal repeats (ITRs) and another that contained two perfectly matched 3′ ITRs, were increased when the mutant transposases were supplied in *trans* in *E. coli*. The use of the perfect repeat transposon with wild type transposase did not result in an increase in transposition frequency in *Ae. aegypti*. However, an improvement in the integrity of the transposition process did occur, as evidenced by a lower rate of recombination events in which the transgene was transferred. An increase in the transpositional activity of the perfect repeat transposon was observed in the mosquito in the presence of either mutant transposase, and in the case of the E264R transposase, the observed increase in transposition frequency was also accompanied by a further improvement in the integrity of transposition. We discuss the possible contributions of these mutant residues to the transposition of the perfect repeat *Mos1* transposon, the implications of these results with respect to the molecular evolution of *Mos1*, and the potential uses of the perfect repeat transposon and mutant transposases for the improvement of *Mos1* mediated germ line transformation of *Ae. aegypti*.

Keywords: *Mos1*; *Mariner*; *Aedes aegypti*; Transposon; Transposable element; Transposition assay

1. Introduction

In an attempt to stem the global rise in the incidence of mosquito-borne diseases, a variety of avenues are being explored in an effort to develop an efficient method of altering the ability of a mosquito species to harbor and/or transmit a specific disease causing pathogen (Ito et al., 2002; Moreira et al., 2002; James, 2003; Dean and Dobson, 2004; Kim et al., 2004; Travanty et al., 2004). The production of transgenic mosquitoes expressing refractory genes represents one approach, and the use of plasmid-borne transposons to produce transgenic insects has been well documented (for a review see O'Brochta and Atkinson, 2004). The *mariner* family of transposons are class II transposable elements that have been identified in a variety of insects, with a partial list including *Drosophila mauritiana* (Jacobson and Hartl, 1985), *Haematobia irritans, Oncopeltus fasciatus, Hylofora cecropia, Apis melifera, Anopheles gambiae, Ephestia cautella* (Robertson, 1993), *Bactrocera tryoni* (Green and Frommer, 2001), *Bombyx mori* (Kumaresan and Mathavan, 2004), *Musca domestica* and *Blattella germanica* (Liu et al., 2004). Other *mariner* elements have identified in non-arthropod invertebrates (Garcia-Fernandez et al., 1995), as well as vertebrates (Morgan, 1995) and plants (Jarvik and Lark, 1998).

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Mariner transposons transpose by a conservative cut-and-paste mechanism whereby the transposase, the coding sequence for which is contained within the transposon, catalyzes the excision of the transposon from one DNA molecule and its subsequent insertion into another DNA molecule at the target dinucleotide, TA. Of the hundreds of *mariners* identified, only *Famar1, Himar1* and *Mos1* are known to be active, and only *Mos1* has been observed to be active in eukaryotic cells (Jacobson and Hartl, 1985; Lampe et al., 1996; Barry et al., 2004). *Mos1*, the best characterized of the three, was originally identified in the genome of *D. mauritiana* (Jacobson and Hartl, 1985). *Mos1* requires no host specific factors for transposition (Tosi and Beverley, 2000), and has been used for transforming a range of organisms including protozoa (Gueiros-Filho and Beverley, 1997); vertebrates (Fadool et al., 1998; Leal et al., 2004); non-arthropod invertebrates (Bessereau et al., 2001); and arthropods (Garza et al., 1991; Lidholm et al., 1993; Coates et al., 1998). Chief among these in the context of efforts to control mosquito-borne diseases is the transformation of *Aedes aegypti* (Coates et al., 1998; Coates et al., 2000), a major mosquito vector of the yellow fever and dengue viruses (Clark and Quiroz Martinez, 2001; Gubler, 2004; Tomori, 2004).

In addition to *Mos1*, the unrelated transposons, *piggyBac* and *Hermes*, have also been used for the successful germ line transformation of *Ae. aegypti*, with rates of transformation reported to be 5–10% (Coates et al., 1998; Jasinskiene et al., 1998; Moreira et al., 2000; Kokoza et al., 2001; Lobo et al., 2002; Adelman et al., 2004). These rates are sufficient for producing a small number of transgenic lines, but due to the costly and time-consuming nature of mosquito transgenesis work, they are suboptimal for the development of gene discovery and analysis technologies for *Ae. aegypti*, such as insertional mutagenesis, which require the production of numerous transgenic lines in order to be effective on a broad scale. Furthermore, none of these transposons have been reported to remobilize to any significant degree in transgenic individuals of this species (Wilson et al., 2003). Remobilization would improve the efficiency of these methodologies by circumventing the need for subsequent microinjection experiments and may also provide a genetic drive mechanism for propagating refractory genes throughout wild populations (Tabachnick, 2003). Unlike *piggyBac* and *Hermes*, *mariner* transposons have been observed to be active in *Escherichia coli*, a characteristic that makes them particularly amenable to mutagenesis studies for the purpose of increasing their transpositional activity.

One study of *Himar1* showed that hyperactive transposase mutants could be produced by random mutagenesis (Lampe et al., 1999). In a previous study of *cis*-acting elements of *Mos1*, we mutated the DNA sequence of the 5′ inverted terminal repeat (ITR) to perfectly match that of the 3′ ITR (Pledger et al., 2004). While this mutant transposon did result in an increase in transpositional activity in *E. coli* using transposase produced *in trans*, an increase was not observed in *Ae. aegypti*. Evidence suggests that the divergence of *mariner* elements during the course of their evolution is due to changes in both ITR and transposase coding DNA sequences (Lampe et al., 2001). As a result of such mutations altering the recognition properties of the transposase–transposon interaction, it is likely that a concomitant down regulation of transposition and/or remobilization might also occur since repeated upregulation over time would likely prove to be deleterious to the host. These observations indicate that efforts to achieve increases in *Mos1* transpositional activity in *Ae. aegypti* might benefit from the simultaneous utilization of mutations in both the *cis*- and *trans*-acting elements of *Mos1* transposition.

Himar1 and *Mos1* appear to be closely related, each sharing a consensus ITR sequence and a characteristic DD(34)D catalytic triad in their transposase amino acid sequences (Tu and Coates, 2004). Given their relatedness, we aligned the *Himar1* and *Mos1* transposase amino acid sequences in order to select those *Mos1* residues which corresponded to the mutated residues in the hyperactive *Himar1* transposases, and the mutations were duplicated in the *Mos1* transposase. These mutant transposases were supplied *in trans*, and their effects were evaluated using a *Mos1* mutant transposon containing the *cis*-acting perfectly matched ITR produced in our previous study (Pledger et al., 2004). The results presented herein indicate that, when used in concert, these mutations can bring about increases in *Mos1* transposition frequency in *Ae. aegypti*. In addition to determining the transpositional activity under these conditions, the character of the integration events were also examined. This analysis demonstrated that mutations in the ITR and transposase coding DNA sequence improved the integrity of the *Mos1* transposition process.

2. Materials and methods

2.1. Mutagenesis

The amino acid sequences of the *mariner* transposases, *Himar1* (GenPept AAM56714) and *Mos1* (GenBank X78906), were aligned with NCBI BLASTP 2.2.10 using the BLOSUM62 matrix at default settings. *Mos1* residues, E137 and E264, corresponded to *Himar1* residues, E137 and H267, and were thus selected for mutation to K and R, respectively, based on the results of the previous Lampe et al. (1999) study. The two mutations were created separately in the *Mos1* transposase open reading frame (ORF) using mutagenic