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Host sequences in *Plasmodium falciparum* and *Plasmodium vivax* genomic DNA: horizontal transfer or contamination artifact?

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Genome sequencing projects have led to new insights into the evolutionary impact of horizontal gene transfer between species [1]. The processes mediating such transfer in eukaryotes could, hypothetically, involve incorporation of host cell DNA into intracellular parasites, viruses and symbionts. Indeed, recent observations show that the *Plasmodium falciparum* genome may be frequently exposed to host sequences, as intra-erythrocytic malaria parasites spontaneously take up and express DNA from host red blood cells that can contain residual nuclear material [2]. We were therefore intrigued by the presence of Alu elements in reported *Plasmodium vivax* and *P. falciparum* DNA sequences [3,4] as well as in several entries in databases from *P. vivax* and *P. falciparum* genome projects (GenBank accession numbers AA550283, AF010561; University of Florida Gene Sequence Tag Project http://parasite.vetmed.ufl.edu/, clone numbers UFL_211PvG06, UFL_214PvC04, UFL_217PvA08, UFL_212PvE06). LINE (L1) sequences have also been identified in some of these databases (University of Florida clone numbers UFL_202PvC10, UFL_211PvE06, UFL_214PvH07, UFL_213PvB11). Both Alu and LINE sequences are abundant interspersed repetitive DNA elements that are characteristic of primate genomes. Since parasite DNA extraction methods are not guaranteed to remove host DNA completely, and it has been shown that host-like sequences in schistosome parasite sequence databases can arise from contamination artifacts [5], we performed experiments to reassess the origin of these Alu and LINE elements.

The reported Alu sequence from *P. vivax* [3] was identified in an expression library that had been constructed with DNA from the blood of an infected patient. This sequence was proposed to be integrated within a gene, *Pv-Alu*, encoding a *P. vivax* antigen. To test this proposal, we performed PCR using oligonucleotide primers specific to the reported sequence to amplify across the junction of the Alu element and the putative parasite gene. These reactions used pure human leukocyte DNA and *P. vivax* DNA prepared from a monkey infection as templates. Results of the amplifications showed a product from human but not parasite DNA (Fig. 1A). Direct sequencing confirmed the product amplified from human DNA to be identical to the reported sequence [3].

We also used the *Pv-Alu* sequence and eight other randomly chosen Alu and L1 DNA fragments identified from the University of Florida *P. vivax* Gene Sequence Tag Project to probe *P. vivax* and *P. falciparum* chromosomal DNAs separated by pulsed field gradient electrophoresis (PFGE). All nine clones hybridized to the same high MW band in the compression region of the *P. vivax* lanes that varied in intensity from preparation to preparation, relative to the intensities of the lower chromosome bands. (Fig. 1B and data not shown). In previous work this band was assumed to be parasite in origin and was described as *P. vivax* chromosome XIV [6]. However, in the light of these results, this assignment appears to be erroneous. We also note that if the repetitive host sequences were indeed true integrants in the parasite genome, they would likely be found at random chromosomal locations. The band to which the sequences hybridize is instead evidently a 'pseudo-chromosome', composed of sheared host material which migrates within the compression region under the PFGE conditions used to separate *P. vivax* chromosomes. In addition, no sequences of confirmed *P. vivax* origin have been found to hybridize to this band ([7] and data not shown). None of the Alu or L1 elements hybridized to gels of separated *P. falciparum* chromosomes, which are from in vitro cultures containing erythrocytes cleared of leukocytes and platelets before use (Fig. 1B).

Southern blots of restricted *P. falciparum* genomic DNA were also probed for the presence of Alu sequences. Under low stringency conditions (3×SSPE at 50°C), these probings did not detect bands that would suggest the presence of Alu-like sequences in the *P. falciparum* genome (data not shown). PCR amplification experiments also failed to confirm other Alu elements [4] that have appeared in *P. falciparum* genome project databases (data not shown).

The above experiments indicate that the human repeat elements cited in the literature and as DNA entries in public sequence databases are most likely contaminants of parasite DNA preparations by host DNA. While this finding does not preclude the possibility that other sequences may have been acquired by horizontal transfer in the evolutionary lineage of *Plasmodium*, caution should be exercised to ensure that such sequences are in fact integrants in the parasite genome. As increasing amounts of data from protozoan genome sequencing projects become available, a more thorough assessment of the issue of horizontal gene transfer in the evolutionary lineage of Plasmodium, raised in [7], should be possible.

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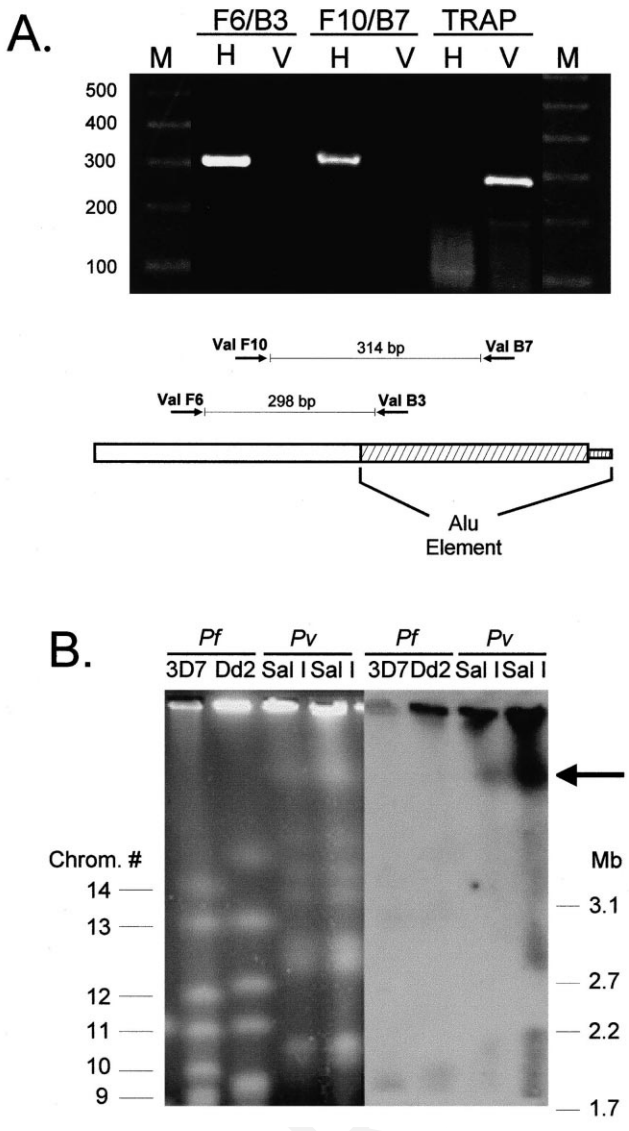

Fig. 1. Evaluation of the presumptive host-like DNA in the *P. falciparum* and *P. vivax* genomes. A: Re-investigation of the Alu element reported to be integrated into the 3′ end of an antigen gene expressed by blood stage *P. vivax* parasites [4]. PCR primers, designed to span the junction of the Alu sequence and the coding region of the putative *P. vivax* gene, yielded products from human but not *P. vivax* genomic DNA. A control reaction is shown using primers specific to the *P. vivax* TRAP gene. A schematic below the gel shows the position of the oligonucleotide primers and the expected size of the products. B: Hybridization of nine different *P. vivax* clones, known to contain sequences with high homology to Alu and L1 repeat families, to PFGE separations of *P.* vivax strain Sal I (*Pv*) and *P. falciparum* strains 3D7 and Dd2 (*Pf*) chromosomes. Chromosomes 9–14 of *P. falciparum* reference clone 3D7 are labeled, the remaining chromosomes having migrated off the gel. The result shown is of hybridization of *P. vivax* clone 202PvF03, but all other clone hybridizations gave similar results. An arrow points to the signal emitted from the top most band found within a zone of compression on the gel. This is most likely sheared contaminating host DNA. Other background signals are as a result of over-exposure of the blot.

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