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Short communication

Evolutionary conservation of RNA editing in the genus *Leishmania*

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Abstract

RNA editing in kinetoplastids is the process by which vital genetic informations are restored through insertion and deletion of uridine residues in coding sequences, particularly those of the mitochondrial pre-mRNA. Mammalian infecting *Leishmania* were not analyzed before for the presence of RNA editing to establish whether the mechanism is still in use in higher lineages of the genus. The Cytochrome Oxidase gene of *Leishmania tarentolae* is known to be edited at its 3′ end with gRNA encoded in the region immediately downstream. We sequenced DNA and cDNA of the COII gene of *Leishmania donovani* and compared those to *Leishmania tarentolae* sequences from the database. The results reveal an insertion of uridines in a manner identical to *L. tarentolae*, leading to restoration of the amino acid sequence with relative conservation of the gRNA region. We conclude that RNA editing as a posttranscriptional mechanism is still conserved within higher evolutionary lineages of the genus *Leishmania*.

Keywords: RNA editing; *Leishmania donovani*; *Leishmania major*; gRNA

1. Introduction

RNA editing (Benne, 1989; Simpson and Shaw, 1989; Stuart, 1989; Landweber and Gilbert, 1994; Maslov et al., 1994) is the process by which the kinetoplastids correct faults in the DNA that affects translation of proteins by insertion and deletion of uridine residues. It is thought to be a primitive genetic phenomenon in evolutionary terms, being significantly reduced in higher evolutionary lineage's within the kinetoplastids with a transition from pan-editing to 5′ editing (Landweber and Gilbert, 1994; Maslov et al., 1994) and largely non-existent in other lower eukaryotes. Editing has neither been hitherto investigated in *Leishmania donovani* one of the major human parasites and a recent evolutionary offshoot of the genus *Leishmania* (Ibrahim and Barker, 2001), nor in any of the kin parasites infecting man and other mammalian hosts. It has been suggested, based on sequence conservation of the editing domain in the ATPase subunit that the mechanism of RNA editing could indeed be conserved between lizard and human *Leishmania* (Brewster and Barker, 1999). RNA editing might have met the fate of posttranscriptional mechanisms that were lost in the course of evolution among members of the genus *Leishmania* e.g. the loss of RNA silencing in the genus *Leishmania* altogether (Ullu et al., 2004). Here we investigate, based on DNA–cDNA sequence alignment the conservation of RNA editing in the cytchrome oxidase II (COII) gene of the human parasite *L. donovani*.

2. Materials and methods

2.1. Parasite DNA extraction

Stationary-phase promastigotes were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). Promastigotes were harvested from culture by centrifugation at 1200 × *g* for 10 min at room temperature and then washed once in phosphate buffered saline (PBS). *Leishmania* total DNA was isolated by centrifugation of 10^8 cultured promastigotes after which the pellet was subjected to detergent lysis (SDS) and phenol chloroform extraction. The DNA was finally suspended

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in TE buffer (pH8) and quantified using a Fluorometer (Hoefer Scientific Instrument).

2.2. RNA extraction and preparation of cDNA

Total RNA was isolated form *L. donovani promastigotes* (*MHOM/SD/63/Khartoum*) grown in RPMI 1640 (Life Technologies) supplemented with 10% FCS, using RNA isolation reagent, RNA wizTM (Ambion). Briefly cells were disrupted and homogenized in RNA wiz. The lysate was then mixed with chloroform and centrifuged causing the homogenate to separate into three phases. The upper aqueous phase containing the RNA was transferred into a clean RNAase-free tube. RNA was precipitated using isopropanol and finally re-suspended in an appropriate amount of RNase free distilled water.

cDNA synthesis was carried out according to SuperScript™ H–Reverse Transcriptase system (GibcoBRL, Life Technologies). cDNA was amplified by PCR using maxicircle primers, Max3, Max2N (Ibrahim and Barker, 2001). RT-PCR products were visualized under UV transilluminator, and the product was purified using Qiaquick kit.

2.3. DNA sequencing

Sequencing was performed using dye termination kits (Amersham) by directly sequencing PCR products using forward or reverse primers as described by Ibrahim and Barker

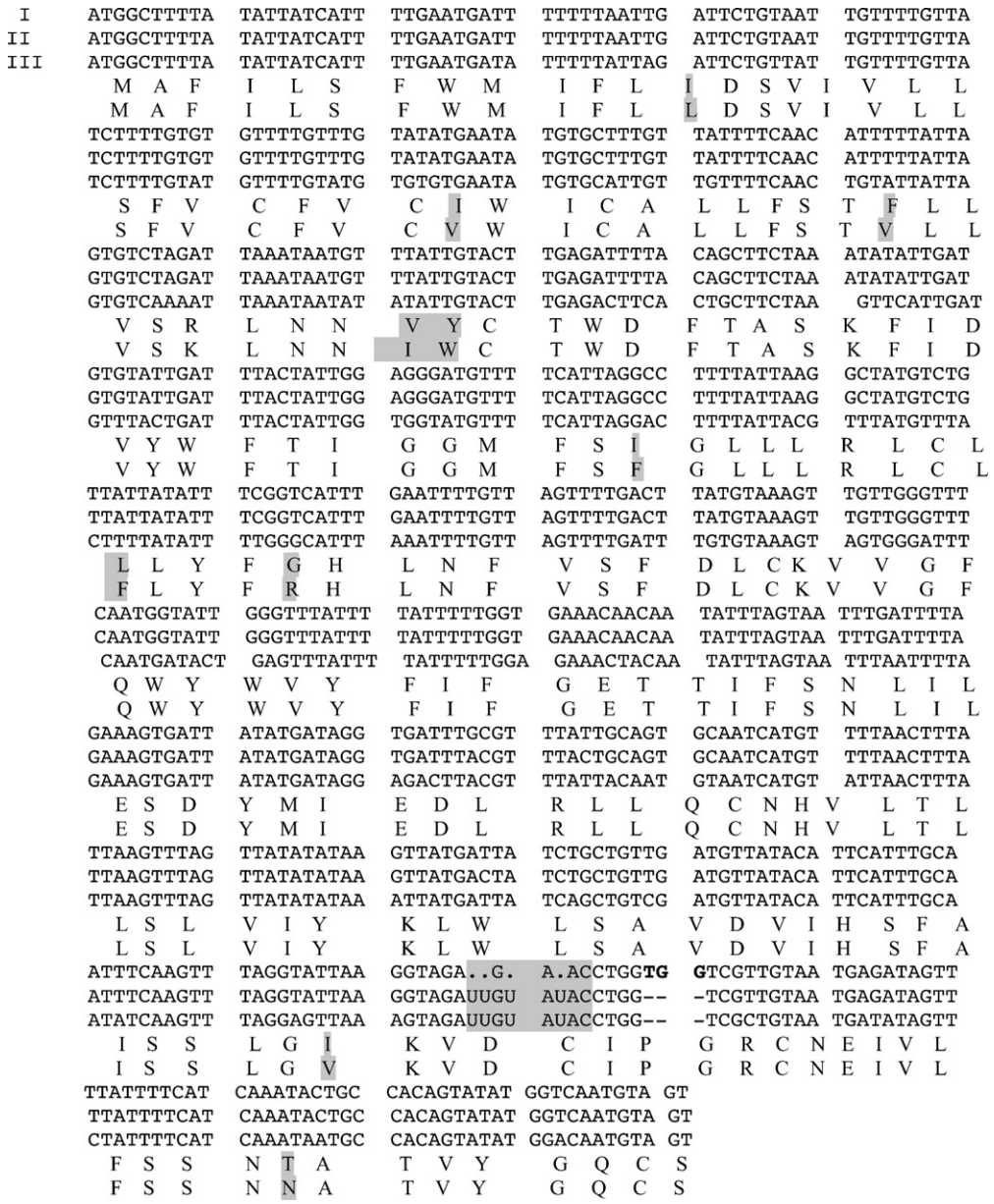

Fig. 1. Alignment of the COII gene sequence from *Leishmania donovani* (lane 1), *L. donovani* cDNA (lane II) and *Leishmania tarentolae* (lane III). Editing is indicated by uridine (u) insertions. The amino acid translation of the *L. donovani* (upper row) and *L. tarentolae* are shown below the DNA sequences. The change in amino acid sequences and edited area are shaded. The boxed area contains three sequences of the gRNA (bottom) and the DNA sequence encoding it in both tarentolae (top) and major donovani (middle).

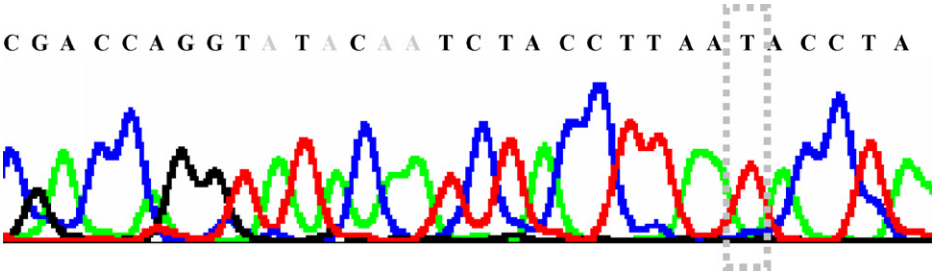

Fig. 2. Chromatogram of the reverse sequence of the 3′ end of the cDNA of the COII of *Leishmania dononvani*. Letters in gray indicate the inserted bases (A or T/U in the sense strand). The dashed box indicates a polymorphism characteristic to *L. donovani* vs *L. tarentolae* (A/T in the donovani replaces a G/C in the tarentolae).

(2001) and an extra reverse primer to cover the 3′ area immediately downstream which encodes the gRNA (5′aatcaaatacacgactcagtcc3′). The reaction products were subsequently sequenced on both directions using LongTower™ System automated sequencer (Visible Genetics) and results were analyzed with Clustal W and the protein translation options within the BioEdit program. Sequence motifs were identified visually.

Leishmania tarentolae, *L. donovan*i, *Leishmania major* and COII DNA and protein sequences were obtained from the public domain using the NCBI blast option. Genbank accession numbers are: M102126 LO7544 COII edited mRNA (*L. tarentolae* edited and pre-edited maxicircle, AF151632, AF287696. AF287688 and EF633106 for the *L. donovani* and *L. major* pre-edited maxicircles.

3. Results and discussion

L. tarentolae the model species for RNA editing (Benne, 1989; Simpson and Shaw, 1989; Stuart, 1989; Landweber and Gilbert, 1994; Maslov et al., 1994), has a known edited domain at the 3′ end of its COII gene which is evolutionary conserved in other kinetoplastidae. This domain, however, has never been shown for human pathogens like *L. major* and *L. donovani*. The fact that extensive editing was found to be greatly reduced along with the evolutionary scale (Landweber and Gilbert, 1994; Maslov et al., 1994), and the absence of similar mechanisms in other primitive eukaryotes may have prompted the speculation of a possible boundary for this phenomenon along the evolutionary scale.

Based on DNA sequence alignment of *L. tarentolae* with *L. donovani* from the database and sequencing and alignment of the COII cDNA of *L. donovani*, we were able to show a 3′ insertion of four uridine bases in *L. donovani* mRNA in a manner identical to that of *L. tarentolae* (nucleotides position 507, 508, 510, 512) (Figs. 1 and 2). The DNA sequence of *L. donovani* COII gene (Ibrahim and Barker, 2001) is 89% similar to that of the *L. tarentolae* and 92.8% identical in amino acid sequence following editing (Fig. 1), a further indication that the genus *Leishmania* is in fact a group of closely related species. To confirm that editing is taking place in a *L. donovani* sequence, Fig. 2 shows the insertion of the four uridines in a sequence with a C → T transition downstream at position 495, that is characteristic to *L. donovani*. The gRNA domain has also been shown to be conserved based on alignment of *L. tarentale* sequences to that of major and donovani except for a single base change (A → G) which apparently does not affect the DNA/gRNA configuration (Pai et al., 2003).

We take the above result as an evidence for the conservation of RNA editing in higher members of the genus *Leishmania* (*L. donovani* and *L. major*). Not surprisingly, given the evolutionary distance separating the donovani/major from tarentolae (Ibrahim and Barker, 2001). It is though necessary to show that this primitive process still persists at tips of the evolutionary lineage's of the genus *Leishmania*, and that it did not meet the fate of other mechanisms like RNA silencing (Ullu et al., 2004) that was lost altogether during a similar evolutionary course. As far as we are aware, this is the first report of RNA editing in a *Leishmania* species other than *L. tarentaloae* with implications to the phylogeny and biology of mammalian parasites of the genus *Leishmania*.

Acknowledgment

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