Published in final edited form as:

J Parasitol. 2005 December ; 91(6): 1492–1495.

Molecular Characterization of a Human BRCA2 Homolog in *Leishmania donovani*

Smita Misra, Mack Hall III, and Gautam Chaudhuri*

Division of Microbial Pathogenesis & Immune Response, Department of Biomedical Sciences, Meharry Medical College, Nashville, Tennessee 37208

Abstract

The breast cancer susceptibility protein BRCA2 is implicated in the DNA double-strand break (DSB) repair pathway through its association with Rad51. It is almost a ubiquitous eukaryotic protein; homologs of the *BRCA2* gene (*BRH2*) have been identified in many mammals, as well as nonmammals. As a part of our quest to understand the DNA damage, repair, and recombination process in the parasitic protozoan, *Leishmania* sp., we have cloned and characterized a *BRCA2* homolog from *Leishmania* sp. (*LBRH2*). *LBRH2* is coded by a single-copy gene (ORF = 3,498 bp) located at the 700-kb chromosome 16. The transcripts in both the promastigotes and the amastigotes are ~3.9 kilonucleotides (knt) in size, corresponding to a protein with a calculated molecular mass of 128 kDa. The primary transcript of the gene is alternatively *trans*-spliced to produce 3 distinct mRNAs with altered folded structures at their 5' ends. This study will contribute toward the understanding of a potential RAD51-mediated DNA recombination/repair pathway in *Leishmania* sp.

Leishmaniasis, a pandemic caused by the several species of *Leishmania*, affects over 12 million people worldwide, with an estimated 2 million new cases each year (Melby, 2002; Desjeux, 2004). Depending on the species involved, symptoms range from the self-curing cutaneous form (e.g., *Leishmania major*) to the fatal visceral form (e.g., *Leishmania donovani*). The parasites are transmitted as promastigotes from the gut of its sandfly vector to the mammalian host macrophages, wherein they transform into amastigotes and proliferate (Handman and Bullen, 2002; Melby, 2002; Desjeux, 2004). Thirty species of sandfly can transmit 20 different species of *Leishmania*. These parasites cause an additional health burden to immunocompromised individuals. As a defense mechanism by macrophages, reactive nitrogen species (RNS) and oxygen species (ROS) are produced to kill the invading cells by damaging their macromolecules, including DNA (Colmenares et al., 2002; Handman and Bullen, 2002; Zambrano-Villa et al., 2002). Despite the presence of RNS- and ROS-catabolizing enzymes in *Leishmania* spp., molecular damages can be caused by these toxic compounds at the DNA level during the routine life cycle process of the parasite. How *Leishmania* spp. survive these oxidative insults, particularly the potential DNA damage that can occur, is not known.

Studies have implicated the involvement of the breast cancer gene, *BRCA2*, in the homologous recombination pathway through its association with Rad51. Loss of *BRCA2* has been shown to lead to a switch from conservative gene conversion to the error-prone single-strand annealing pathway (Jasin, 2002; Keen and Davidson, 2003). Some evidence exists for

a role of *BRCA2* in transcriptional regulation; specifically, *BRCA2* is implicated in DNA break repair (DSB) by homologous recombination (Jasin, 2002; Keen and Davidson, 2003).

The repair of DSBs by homologous recombination is essential in maintaining genome integrity and for the accurate segregation of chromosomes during cell division (Narod and Foulkes, 2004). Defects in DNA repair pathways are associated with several cancer-prone syndromes in humans (Jasin, 2002; Keen and Davidson, 2003). Therefore, understanding the role of a *BRCA2* homolog in the mechanisms of DSB repair will be important in understanding these human pathologies. Despite numerous studies to understand the biochemistry of human *BRCA2* protein, a clear determination of its role in the mechanism of DSB repair remains elusive. In part, this could be because of the complexity of the human *BRCA2* protein (3,418 amino acids) and lack of a simple ortholog in well-studied systems like *Saccharomyces cerevisiae*, *Drosophila* sp., and *Caenorhabditis elegans*. Homologs of human *BRCA2* protein have been identified in many other mammals, birds, fungi, and plant cells; the sizes of some of these proteins are approximately one-third that of human *BRCA2* (Lo et al., 2003).

In human breast cells, Rad51 binds to *BRCA2* with the BRC repeats. Human *BRCA2* protein has 8 BRC repeats (see Fig. 1A). Each BRC repeat is 30–80 amino acids in length and located within exon 11 of human *BRCA2* (Bork et al., 1996; Bignell et al., 1997). These repeats have been demonstrated by yeast 2-hybrid analysis to be both necessary and sufficient for stable binding of human *BRCA2* to RAD51 (Wong et al., 1997; Chen et al., 1998; Katagiri et al., 1998). Nevertheless, despite the identification of *BRCA2*–RAD51 protein–protein interactions, the contribution of these interactions to the tumor suppressor functions of *BRCA2* remains uncertain.

We report here the molecular cloning and characterization of the *BRCA2* homolog, *LBRH2*, from *L. donovani*. The deduced amino acid sequence from *LBRH2* has different domains conserved in other eukaryotic *BRH2*, including the BRC repeats. One of the major *BRCA2* binding proteins, RAD51, has already been characterized from *L. major* (McKean et al., 2001), and we also have cloned LRAD51 from *L. donovani* (GenBank AY395708). Studies of these 2 proteins will lead to a better understanding of the functionality of *BRCA2*.

Leishmania donovani (MHOM/IN/80/DD8) promastigotes were cultured in M199 medium supplemented with 10% heat-inactivated fetal bovine serum (HIFBS) at 26 C with moderate shaking (Mishra, Bennett, and Chaudhuri, 2001; Mishra, Porter-Kelly et al., 2001). The in vitro culture of the axenic amastigotes was done in M199 medium with 25% HIFBS and 10 mM sodium succinate, titrated to pH 5.5, at 37 C (5% CO₂) (Mishra, Bennett, and Chaudhuri, 2001; Mishra, Porter-Kelly et al., 2001).

With the use of *Ustilago maydis* *BRH2* (GenBank AY124376) amino acid sequence (Kojic et al., 2002) as a query, we initially obtained the putative *BRH2* ORF for *L. major* from the Sanger Center *L. major* gene database (http://www.sanger.ac.uk/cgi-bin/blast/submitblast/L_major). We designed primers (5'-GAGAATTCCATGAAGCCGATTGCCTGTCCG-3'/5'-GAGAATTCCTAGTCGACGGGAAGCTCCGC-3') and amplified *L. donovani* *BRH2* ORF from its genomic DNA (*LBRH2*, GenBank AY395707). The deduced amino acid sequence was used for further analysis. We used ClustalW (<http://clustalW.genome.jp/>) for the construction of the dendrogram. PSORTII analysis (<http://psort.nibb.ac.jp/form2.html>) was used for the study of the organelle targeting.

To determine the gene copy number for *LBRH2* in *L. donovani*, we did genomic Southern blot analysis. Genomic DNA (5–10 µg) was digested with different restriction endonucleases. The restriction endonucleases chosen do not have a site in the DNA used as

probe (Fig. 3A). The digests were resolved on a 0.8% agarose gel and transferred to nylon membrane in denaturing buffer (Schleicher and Schuell, Keene, New Hampshire) following their recommended protocols. The blots were probed with a ^{32}P -labeled *LBRH2* ORF fragment amplified from genomic DNA with the primer set 5'-CGATTGCCTGTCCGCATTG-3'/5'-CGCTCGCACGCAGTTGTAG-3' following standard protocols (Sambrook and Russell, 2001).

We performed pulsed-field agarose gel electrophoresis (Bastien et al., 1992) for the chromosomal localization of *LBRH2* in *L. donovani*. Late log phase *L. donovani* promastigotes were harvested and made into blocks of agarose. The agarose blocks were transferred to a lysis mixture containing 0.5 M sodium *N*-laurylsarcosinate and 2 mg proteinase K/ml. This mixture was then incubated at 45 C for 48 hr. The agarose blocks were washed with 1× TBE twice and placed in the slots of a 1% agarose gel. Chromosomal DNAs were electrophoresed through the gel by a CHEF-DRII pulsed-field electrophoresis system from Bio-Rad (Norcross, Georgia) at the switch time of 60–90 sec for 17 hr, followed by a 90–120-sec switch time for 7 hr at 6 V/cm and at 6 C. The DNA in the gel was partially depurinated before transfer onto the membrane by soaking the gel twice in 0.1 N HCl for 20 min with gentle shaking at room temperature. The transfer of the DNA was done in 0.4 N NaOH and 1.5 M NaCl solution for 24 hr with upward capillary rise (Sambrook and Russell, 2001). The membrane was neutralized with 0.5 M Tris.Cl, pH 7.0, for 5 min, followed by 2× SSC (0.3 M NaCl, 30 mM sodium citrate pH 7.4) wash. The blot was hybridized with ^{32}P -labeled *LBRH2* ORF fragment (Fig. 3A) as a probe, as described above.

The expression and transcript size of *LBRH2* in *L. donovani* promastigotes and amastigotes was determined by northern blot analysis. RNA was isolated from *L. donovani* promastigotes and axenic amastigotes with Trizol reagent (Invitrogen, Carlsbad, California) following the manufacturer's protocol. Total RNA was fractionated on a formaldehyde–agarose (1%) gel, transferred to a nylon membrane, and probed with the *LBRH2* ORF fragment (Fig. 3A) as above. RNA levels were evaluated by spot densitometry analysis with an Alpha Imager (Alpha Innotech Corporation, San Leandro, California) and were normalized against the intensity of ethidium bromide–stained ribosomal RNA bands.

To amplify the 5'- and 3'-untranslated region (UTR) sequences of *LBRH2* mRNAs, we used the standard reagents and protocols provided by the supplier (Invitrogen). For 5' rapid amplification of cDNA ends (5'-RACE), we made first-strand cDNAs from total RNA with random hexamers as primers and used 5'-anchored oligo(dT) as the primer for 3'-RACE. In both cases, we used Superscript II (Invitrogen) as the reverse transcriptase. Because all *Leishmania* sp. mRNAs have a 39-nucleotide (nt) minixen sequence at their 5' ends, the minixen-specific sense primer (MEX2: 5'-GGATCCAGTTTCTGACTTTATTG-3') was generated from the 39-nucleotide spliced leader sequence (Chaudhuri, 1997). For the amplification of the 5'-UTR sequence, we used MEX2 as a sense primer and an *LBRH2* gene-specific nested pair of antisense primers, 5'-TCGCTCTGCCACTTTTTTG-3' and 5'-ATGGCATCGGAGACTTTG-3'. The 3' end of the *LBRH2* cDNA was amplified following 3'-RACE amplification protocols (Invitrogen). For 3'-RACE analysis, AUAP (5'-GGCCACGCGTCGACTAGTAC-3') was used as the universal primer with the nested pair of sense primers from *LBRH2* ORF (5'-ACCTGAAGGAGGCTTTGGAG-3' and 5'-GCGATGAGGACAATGAGTG-3'). Amplified PCR products generated by 5'- and 3'-RACE were cloned into the pCR4.0 vector (Invitrogen), and the inserts of several clones were sequenced.

A *Leishmania* genome database search revealed that both *L. major* and *L. donovani* *BRH2* ORFs code for a 1,165–amino acid protein. There is 93% identity between these 2 protein

sequences. PSORTII analysis indicated that the potential for subcellular localization of the LBRH2 protein is nuclear. In contrast to the nuclear localization signal of human *BRCA2*, which is located at the C-terminus of the protein, the putative nuclear localization signal, AGRGRKG, of *LBRH2* is located at the N-terminus (residues 42–48; Fig. 1A, B). One of the hallmarks of *BRCA2* proteins is the BRC repeat, the primary site for RAD51 binding. *LBRH2* has 2 BRC repeats (residues 109–130 and 147–168) in contrast to the human *BRCA2*, which has 8 BRC repeats (Fig. 1A). BRC repeats in *LBRH2* are very similar to each other and to the human counterpart. *BRCA2* has been shown to interact with DSS1, a protein that was originally identified in association with the developmental disorder of split hand/split foot malformation, and this interaction is evolutionarily conserved. DSS1 is shown to be involved in an error-prone repair mechanism (Kojic et al., 2003). A DSS1 homolog in *Leishmania* sp. is yet to be identified. *BRCA2* interacts with DSS1 through the DNA/DSS1 binding domain (DBD). DBD in human *BRCA2* consists of a helical domain (HD), followed by oligobinding (OB) domain 1, the OB2/Tower domain, and the OB3 domain. By ClustalW alignment with *U. maydis* BRH2 protein, we found domains homologous to HD (residues 370–420), OB1 (residues 421–547), and OB2/Tower domains (residues 548–749) in *LBRH2* protein (Fig. 1A, B). Like the *U. maydis* BRH2 (Kojic et al., 2003), the amino acid sequence that follows the OB2/Tower domain in *LBRH2* has no significant similarity with the OB3 domain of human *BRCA2*. Functional analysis of these putative domains by deletion and site-directed mutagenesis will reveal their role, if any, in DNA recombination and DSB repair in *Leishmania* sp.

As one of the earliest diverging eukaryotes with a mitochondrion, *L. donovani* and other trypanosomatids occupy a unique position in evolution. The evolutionary divergence of trypanosomatids from higher eukaryotes suggested that the *L. donovani* and *Trypanosoma brucei* *BRH2* genes could be ancestral to *BRCA2* of higher eukaryotes. To test this hypothesis, we conducted phylogenetic analyses to determine the evolutionary relationship among the *L. donovani* and other *BRH2* homologs. Phylogenetic reconstructions revealed that the *Leishmania* sp. *BRH2* form a distinct clade with *T. brucei* *BRH2*, which is closer to the mammalian *BRCA2* than those of fungi and plants (Fig. 2).

Leishmania sp. genomic DNA digested with restriction endonuclease that do not have sites on the homologous probe for *LBRH2* showed only a single band (Fig. 3B), indicating a single copy of the gene in the genome. This information will be useful for designing the gene knockout experiment to understand the role of *BRCA2* in the cellular paradigm. Pulsed-field gel electrophoresis revealed that the *LBRH2* gene was found on chromosome number 16 in *L. donovani* (Fig. 3C), which supports *L. donovani* genome databank observations.

Northern blot analysis of RNA isolated from *L. donovani* promastigotes and axenic amastigotes showed the expression of an ~3.9-knt *LBRH2* mRNA in both forms (Fig. 4A). The amastigotes appear to have 1.5- to 2-fold higher levels of *LBRH2* transcripts than in the promastigotes (Fig. 4B). Amastigotes perhaps live in a more stressful environment than the promastigotes; thus, an increased level of *BRCA2* in the cell might be warranted. Further analysis of the regulation of expression of *LBRH2* is underway.

The 5'-RACE showed that the majority (5 of 10 clones) of *LBRH2* mRNA 5'-UTR in *L. donovani* is 233 nt, including the 39-nt miniexon sequence (Fig. 5A). We found alternative *trans*-splicing in the biosynthesis of this transcript. This process yields 223-nt (3 of 10 clones) and 178-nt (2 of 10 clones) 5'-UTR sequences of the mRNA (Fig. 5A). The generation of multiple messages for each *LBRH2* occurred through the choice of alternative *trans*-splicing with the attachment of the miniexon at 1 of several 3' splice acceptor sites. We did not find any evidence for alternative 3' polyadenylation, and the length of the 3'-UTR of *LBRH2* mRNA in *L. donovani* is 86 nt (Fig. 5B). Mfold analysis (Zuker, 2003) of

the 3 different 5'-UTR sequences to determine their best possible secondary structures that will be the energetically most stable conformations, showed distinct differences. The structure for the longest 5'-UTR is more relaxed, having a bubble, but it becomes a little more compact with the shortening of the UTR to 223 nt by loosing the bubble initially present in the longest form, although the structure compacts further in the shortest 5'-UTR. Alternative *trans*-splicing of mRNA precursors are known to occur in trypanosomes to regulate the expression of different genes (Vassella et al., 1994; Manning-Cela et al., 2002). The effect of the differential length and the structure of the 5'-UTR on *LBRH2* gene expression and stability is under study.

Acknowledgments

We thank Manish K. Tripathi for reading the manuscript and for his valuable comments. This work was supported in part by the NIH grant 5R01AI42327-03 and 3 SO6 GM008037-33S1 to G.C.

LITERATURE CITED

- Bastien P, Blaineau C, Pages M. *Leishmania*: Sex, lies and karyotype. *Parasitology Today*. 1992; 8:174–177. [PubMed: 15463609]
- Bignell G, Micklem G, Stratton MR, Asworth A, Wooster R. The BRC repeats are conserved in mammalian BRCA2 proteins. *Human Molecular Genetics*. 1997; 6:53–58. [PubMed: 9002670]
- Bork P, Blomberg N, Nilges M. Internal repeats in the BRCA2 protein sequence. *Nature Genetics*. 1996; 13:22–23. [PubMed: 8673099]
- Chaudhuri G. Scavenger receptor-mediated delivery of anti-minixon antisense phosphorothioate oligonucleotide to *Leishmania*-infected macrophages: Selective and efficient elimination of the parasite. *Biochemical Pharmacology*. 1997; 53:385–391. [PubMed: 9065742]
- Chen PL, Chen CF, Chen Y, Xiao J, Sharp ZD, Lee WH. The BRC repeats in BRCA2 are critical for RAD51 binding and resistance to methyl methanesulfonate treatment. *Proceedings of the National Academy of Sciences USA*. 1998; 95:5287–5292.
- Colmenares M, Kar S, Goldsmith-Pestana K, McMahon-Pratt D. Mechanisms of pathogenesis: Differences amongst *Leishmania* species. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2002; 96:3–7.
- Desjeux P. *Leishmaniasis*. *Nature Reviews in Microbiology*. 2004; 2:692–693.
- Handman E, Bullen DV. Interaction of *Leishmania* with the host macrophage. *Trends in Parasitology*. 2002; 18:332–334. [PubMed: 12377273]
- Jasin M. Homologous repair of DNA damage and tumorigenesis: The BRCA connection. *Oncogene*. 2002; 21:8981–8993. [PubMed: 12483514]
- Katagiri T, Saito H, Shinohara A, Ogawa H, Kamada N, Nakamura Y, Miki Y. Multiple possible sites of BRCA2 interacting with DNA repair protein RAD51. *Genes, Chromosomes and Cancer*. 1998; 21:217–222. [PubMed: 9523196]
- Keen JC, Davidson NE. The biology of breast carcinoma. *Cancer*. 2003; 97:825–833. [PubMed: 12548582]
- Kojic M, Kostrub CF, Buchman AR, Holloman WK. BRCA2 homolog required for proficiency in DNA repair, recombination, and genome stability in *Ustilago maydis*. *Molecular Cell*. 2002; 10:683–691. [PubMed: 12408834]
- Kojic M, Yang H, Kostrub CF, Pavletich NP, Holloman WK. The BRCA2-interacting protein DSS1 is vital for DNA repair, recombination, and genome stability in *Ustilago maydis*. *Molecular Cell*. 2003; 12:1043–1049. [PubMed: 14580353]
- Lo T, Pellegrini L, Venkitaraman AR, Blundell TL. Sequence fingerprints in BRCA2 and RAD51: Implications for DNA repair and cancer. *DNA Repair*. 2003; 2:1015–1028. [PubMed: 12967658]
- Manning-Cela R, Gonzalez A, Swindle J. Alternative splicing of LYT1 transcripts in *Trypanosoma cruzi*. *Infection and Immunity*. 2002; 70:4726–4728. [PubMed: 12117992]

- McKean PG, Keen JK, Smith DF, Benson FE. Identification and characterization of a RAD51 gene from *Leishmania major*. *Molecular and Biochemical Parasitology*. 2001; 115:209–216. [PubMed: 11420107]
- Melby PC. Recent developments in leishmaniasis. *Current Opinion in Infectious Diseases*. 2002; 15:485–490. [PubMed: 12686880]
- Mishra M, Bennett JR, Chaudhuri G. Increased efficacy of antileishmanial antisense phosphorothioate oligonucleotides in *Leishmania amazonensis* overexpressing ribonuclease H. *Biochemical Pharmacology*. 2001; 61:465–474.
- Mishra M, Porter-Kelley J, Singh PK, Bennett JR, Chaudhuri G. Enhanced activity of antisense phosphorothioate oligos against *Leishmania* amastigotes: Augmented uptake of oligo, ribonuclease H activation, and efficient target intervention under altered growth conditions. *Biochemical Pharmacology*. 2001; 62:569–580. [PubMed: 11585054]
- Narod SA, Foulkes WD. BRCA1 and BRCA2: 1994 and beyond. *Nature Reviews Cancer*. 2004; 4:665–676.
- Sambrook, J.; Russell, DW. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, New York: 2001. p. 1.1-18.125.
- Vassella E, Braun R, Roditi I. Control of polyadenylation and alternative splicing of transcripts from adjacent genes in a procyclin expression site: A dual role for polypyrimidine tracts in trypanosomes? *Nucleic Acids Research*. 1994; 22:1359–1364. [PubMed: 8190625]
- Wong AKC, Pero R, Ormonde PA, Tavtigian SV, Bartel PL. RAD51 Interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene *brca2*. *Journal of Biological Chemistry*. 1997; 272:31941–31944. [PubMed: 9405383]
- Zambrano-Villa S, Rosales-Borjas D, Carrero JC, Ortiz-Ortiz L. How protozoan parasites evade the immune response. *Trends in Parasitology*. 2002; 18:272–278. [PubMed: 12036742]
- Zuker M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research*. 2003; 31:3406–3415. [PubMed: 12824337]

Analysis of the amino acid sequence of LBRH2. **(A)** Domain structures of human BRCA2 (HsBRCA2) and the BRCA2 homolog from *Leishmania* (LBRH2) protein showing the nuclear localization signals (NLS), BRC repeats, and DSS1/DNA binding domains (DBD). **(B)** Amino acid sequence of the LBRH2 protein showing the nuclear localization signal (residues 42–48), BRC domains (LBRC1, residues 109–130; LBRC2, residues 147–168), helical domain (residues 370–420), and oligobinding domains (OB1, residues 421–547, OB2/Tower, residues 548–749). LBRH2 lacks the OB3 domain that is present in HsBRCA2.

Analysis of the amino acid sequence of LBRH2. **(A)** Domain structures of human BRCA2 (HsBRCA2) and the BRCA2 homolog from *Leishmania* (LBRH2) protein showing the nuclear localization signals (NLS), BRC repeats, and DSS1/DNA binding domains (DBD). **(B)** Amino acid sequence of the LBRH2 protein showing the nuclear localization signal (residues 42–48), BRC domains (LBRC1, residues 109–130; LBRC2, residues 147–168), helical domain (residues 370–420), and oligobinding domains (OB1, residues 421–547, OB2/Tower, residues 548–749). LBRH2 lacks the OB3 domain that is present in HsBRCA2.

Figure 2.

Dendrogram showing the relationships between different BRCA2-like proteins. The accession numbers for the proteins analyzed are as follows: *Leishmania donovani*, AAQ96330; *Trypanosoma brucei*, CAB95357; *Felis catus*, BAC75821; *Homo sapiens*, CAI13195; *Rattus norvegicus*, NP_001007718; *Oryza sativa*, NP_909396; *Canis familiaris*, NP_001006654; *Gallus gallus*, NP_989607; *Mus musculus*, AAK57537; *Arabidopsis thaliana*, NP_195783; *Caenorhabditis elegans*, AAR98640; *Ustilago maydis*, XP_400815; *Anopheles gambiae*, XP_308734.

Figure 3.

Genomic structure of *LBRH2* in *Leishmania donovani*. **(A)** The partial restriction map of *LBRH2* coding sequence showing the region from which the probe DNA was amplified and used for Southern and northern blot hybridization analyses. **(B)** Autoradiogram showing genomic Southern blot analysis to determine the gene copy number for *LBRH2* in *L. donovani*. Restriction endonuclease that was used to digest total genomic DNA are shown at the top of each lane. **(C)** Chromosomal location of *LBRH2* in *L. donovani*. Left panel: photograph of the EtBr-stained pulsed-field agarose gel electrophoresis; right panel: corresponding autoradiogram.

Figure 4.

Analysis of the levels of *LBRH2* mRNA in *Leishmania donovani* promastigotes and amastigotes. (A) Northern blot analysis of total RNA. Left panel: photograph of the EtBr-stained formaldehyde agarose gel showing 3 rRNA bands. Right panel: corresponding autoradiogram. RNA (10 µg) isolated from promastigotes (left) and axenic amastigotes (right) were analyzed. (B) Spot densitometric analysis of the northern blot autoradiograms. Several autoradiograms were scanned in a gel documentation system, and the intensity of the *LBRH2* mRNA band was normalized with that of the corresponding uppermost rRNA band. The plot indicates the relative quantities of *LBRH2* mRNA in *L. donovani* promastigotes and amastigotes. Results are mean ($n = 5$) $\pm$ SE.

(A) Nucleotide sequences of the 5'-RACE products

AACUAAAGCUAUAUAAGUAUCAGUUUCUGUACUUUAUUG/CACUCCUAG/CUGC
 CGCUGCCUCUGCCUGCGACACGCGUACAGCCAAGCGUCAG/CGGGUGGGGACACU
 GCACGCAUCUCUGAGUGAGGGAGCUGCGUGCCCGGGUGGACGCCCUGCCACCU
 ACUGCGUGCGUUUCCAUACCUAGAGUCUCGAAGAGGCAGACGCGCGGUGUGUCU
 UGAUACACCGCCGCGaug

(B) Nucleotide sequence of the 3'-RACE product

uagCGUUUCUGCAUUGCAGCCACUGGGCACAAAAACCGGCGACAGCGGUCAUUA
 CGAGGGAGAGUCCACGUGGAAGCUUCCGCC**AAAAAAAAAAAAAAAA**

Figure 5.

Determination of the 5'- and 3'-untranslated regions (UTRs) of *LBRH2* mRNAs from *L. donovani*. **(A)** Nucleotide sequence of the 5'-UTR of *LBRH2*. The miniexon sequences are shown bold and underlined. The start and the stop codons are shown in lowercase and are bold. There are 3 splice variants of the *LBRH2* mRNA. Different *trans*-splicing sites are shown by the back slash (/). **(B)** The 3'-UTR sequence of *LBRH2* mRNA in *L. donovani*.