Protist, Vol. 166, 457–467, September 2015
http://www.elsevier.de/protis
Published online date 22 July 2015

Protist

ORIGINAL PAPER

A DEVH-box RNA Helicase from *Leishmania braziliensis* is Associated to mRNA Cytoplasmic Granules

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Submitted December 17, 2014; Accepted July 6, 2015
Monitoring Editor: Michael L. Ginger

RNA helicases are ubiquitous enzymes that participate in almost all aspects of RNA processing, including RNA and RNA-protein complex remodelling. In trypanosomatids, which post-transcriptionally regulate gene expression, the formation of different kinds of ribonucleoprotein granules under stress conditions modulates the parasite's RNA metabolism. This paper describes the isolation of a putative DEVH-box RNA helicase produced by promastigotes of *Leishmania braziliensis*. Using a Cy3-labelled dT_{30} oligo, FISH showed the localization of this protein to mRNA granules under starvation stress conditions. The central region of the protein was shown to be responsible for this behaviour.

Key words: Trypanosomatids; *Leishmania*; RNA helicase; mRNA granules.

Introduction

Parasitic protozoa of the genus *Leishmania* are responsible for a broad spectrum of diseases known as leishmaniasis, which worldwide cause 20,000-40,000 deaths every year (Alvar et al. 2012). These parasites have complex life cycles with two stages: an extracellular stage involving promastigotes carried by sandflies, and an intracellular, proliferative, amastigote stage that occurs in the cells of vertebrate hosts (Molyneux and Killick-Kendrick 1987). They therefore have to adapt to different environments, and may find themselves subject to heat shock (e.g., when the promastigote passes between vector and host), or hyperosmolarity or oxidative stress (e.g., when the promastigote enters a host cell).

The regulation of gene expression in both stages of the *Leishmania* life cycle is post-transcriptional (Clayton and Shapira 2007). The demands imposed by stress conditions might therefore be met by the parasite sequestering untranslated mRNA and storing it in granules along with other proteins involved in splicing, transcription, adhesion and signalling (Kedersha and Anderson 2007). mRNA granule formation has been described in yeasts, metazoans and protozoans (Anderson and Kedersha 2009), including trypanosomatids such as *Leishmania* spp., *Trypanosoma brucei* and

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http://dx.doi.org/10.1016/j.protis.2015.07.002
1434-4610/

Trypanosoma cruzi (Cassola 2011; Zinoviev et al. 2012). Indeed, several trypanosomatids have been reported to produce different kinds of cytoplasmic granules under different stress conditions (Cassola et al. 2007; Holetz et al. 2007; Kramer et al. 2008; Kramer 2014).

RNA-binding proteins (RBPs) play a critical role in the regulation of mRNAs - both free in the cellular environment and sequestered in granules (Fernández-Moya and Estévez 2010) - via their participation in the formation of mRNA-protein complexes (mRNPs). RNA helicases are very likely involved (Hooper and Hilliker 2013). These enzymes unwind the secondary structure of RNA and/or displace attached proteins from RNA using energy released by the hydrolysis of ATP (Linder 2006). Most belong to the SFII superfamily and are classified into families depending on the consensus sequence of the conserved motif II, i.e., DEAD, DEAH or DEXH (Gorbalenya et al. 1989). The helicase central region (HCR) contains eight conserved motifs with different functions. Motifs I and II are required for ATP binding and hydrolysis, motifs III and V are primarily involved in the coordination between NTPs and the nucleic acid binding site, and motif VI is involved in ATP hydrolysis and RNA unwinding (Fairman-Williams et al. 2010; Gross and Shuman 1996; Kim et al. 1997). The HCR is flanked by N- and C- terminal domains or halves which are often larger than the HCR itself. These flanking regions are thought to be crucial in determining the subcellular localization of the enzyme, and thus the promotion of its interactions with other proteins, as well as its recruitment into different complexes (Fairman-Williams et al. 2010; Jankowsky 2011). For example, the N-terminal of the DEAH-box RNA helicase RHAU is necessary for its accumulation in stress granules (SGs) (Chalupníková et al. 2008).

Different RNA helicases associate constitutively or transiently with mRNA granules such as P-bodies and/or SGs in different organisms, and are involved in the translation initiation and/or repression and mRNA decay pathways (Hilliker 2012). In the trypanosomatid *T. cruzi*, the DEAD-box helicase DHH1 co-localizes with mRNA granules (Cassola et al. 2007) that vary in number and location depending on the life cycle status and nutritional condition of the parasite (Holetz et al. 2010). This protein has also been found in P-bodies providing stability to developmentally regulated mRNAs (Kramer et al. 2010).

The present work identifies a putative DEVH-box RNA helicase produced by promastigotes of *L. braziliensis* that localizes to cytoplasmic mRNA granules under different stress conditions. The involvement of the HCR in the formation of these granules is revealed.

Results and Discussion

Isolation and Characterization of the *L. braziliensis* Putative DEVH-box RNA Helicase LbrDEVH1

The RNA helicase LbrM.22.1400 produced by *L. braziliensis* MHOM/BR/75/M2904 (sequence provided by http://tritypdb.org) was amplified with specific primers designed using the genomic DNA of *L. braziliensis* MHOM/BR/75/M2903 as template. An amplicon of 3252 bp was directly cloned into the pGEM T easy vector. Sequencing of both insert strands plus BLAST analyses confirmed the identity of the *L. braziliensis* LbrDEVH1 helicase coding sequence (deposited in the European Nucleotide Archive under accession number HG792877).

LbrDEVH1 and its syntenic counterparts in *L. infantum* (LinJ.22.1350), *L. major* (LmjF.22.1500), *L. donovani* (LdBPK_221350.1) and *L. mexicana* (LmxM.22.1500) exhibited extensive sequence conservation. Therefore, these proteins may well play identical roles in these different species. The sequence from *L. braziliensis* M2903 had a 456 nt-long region at the 5′end absent from all other sequences. Strong similarities were also seen between the LbrDEVH1 helicase nucleotide and protein sequence and its homologues in other trypanosomatids (73% with that of *Crithidia fasciculata* [CfaC1.24.1700], 50% with that of *T. cruzi* [TcCLB.506861.10], and 47% with that of *T. brucei* [Tb927.6.740]). The differences of the sequences between species due to the evolutionary separation (Peacock et al. 2007) was corroborated with a phylogenetic tree (Fig. A.1).

When compared to DHX36 ATP-dependent RNA helicase from *Homo sapiens*, however, the similarity was just 36%. The human orthologue protein is involved in the regulation of RNA stability and gene transcription (Booy et al. 2012; Tran et al. 2004; Vaughn et al. 2005), the localization in dendrites of neuronal precursor miR-134 (Bicker et al. 2013), and is an SG-associated protein (Chalupníková et al. 2008).

The deduced amino acid sequence for LbrDEVH1 helicase (a putative DEVH-box RNA helicase) showed four domains: the R3H domain (predicted to bind to ssDNA or ssRNA in a sequence-specific manner [residues 1-28]), the DEXDc or DEAD-like helicase domain that