

The mRNA coding for the nucleosomal protein H2A of *Leishmania* is polyadenylated and has stem-loops at the 3′ end

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In the present report we describe the cloning and characterization of a cDNA coding for the histone H2A from *Leishmania donovani infantum* after probing a library of cDNA in lambda gt 11 with a serum from a dog with visceral leishmaniasis. The sequence analysis of one of these clones, called cL71, shows extensive sequence similarity with the histone H2A DNA sequences of other organisms (1) . The insert of cL71 clone is 699 nucleotides in length and the ORF coding for the histone H2A starts in nucleotide 81 and ends at position 479. The coding region is 67.2% GC-rich. Although a preferential use for GC-rich codons has been noted for several histone DNA sequences (2), this preference is quite marked for *Leishmania* H2A with 118 of the 132 codons (90%) having the maximum possible GC content. The *Leishmania* H2A is 132 amino acids in length with a deduced molecular weight of 13,892 daltons and an isoelectric point of 11.66. This protein shows an amino acid sequence similarity of about 70% with other H2A histone sequences from different organisms (1). This high level of sequence conservation is probably reflecting the universality of function of this protein, relative to cell division and DNA packing, in eukaryotic organisms.

We have observed that all the *Leishmania* H2A mRNAs are polyadenylated since after oligo-dT purification of *Leishmania* mRNA, the cDNA probe hybridizes only with the poly(A)+ fraction (data not shown). In contrast, most of the histone mRNAs from eukaryotic cells are not polyadenylated (1), with the exception of the variant histones of higher organisms (3, 4), all histone mRNAs from yeast cells (5), and the H3 and H4 histone mRNAs from many plants (6). A characteristic feature of histone mRNAs, also present in *Leishmania* H2A mRNAs, is a sequence of dyad symmetry located at the 3′ terminus. This sequence has been implicated in the processing mechanism generating the 3′ ends and the stability of histone mRNAs (7). After a search for inverted repeats into the cL71 sequence, we found three structures with potential to form stem-loops similar in secondary structure to those described for other eukaryotic histone mRNAs (Fig. 1). Hairpins A and B are mutually excluding because almost the same nucleotides are involved in their formation. Hairpin C contains a 7 bp long stem that possesses a mismatch at position 4 instead of the 6 bp perfect stem observed in other eukaryotes (7). Interestingly, the stemloop C ends just at the site of polyadenylation. Thus, since several histone mRNAs are either polyadenylated or 3′ processed at the stem-loop position depending on the phase of the cell cycle (3) we think that it would be of interest to analyze whether the stem loop plays any significant role in the expression and stability of the *Leishmania* H2A mRNA during the different stages of the parasite.

At present, studies on chromatin structure and composition in Trypanosomatids are scanty, and most of them refer to physicochemical characterization of histone-like proteins (8). Since, to date, only the sequence of histone H2B from *L.enriettii* has been determined (9), this is the first report of the molecular characterization of a nucleosomal protein in trypanosomatids belonging to the H2A family.

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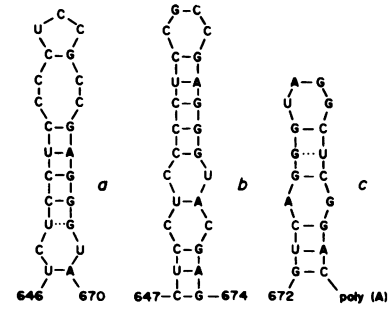

Figure 1. Potential stem-loops at the 3′ termini of *L.d.infantum* histone H2A mRNA. The G-U base pairs are indicated by dotted lines. The numbers indicate the position of each potential stemloop into the nucleotide sequence of cL71 cDNA.