SHORT COMMUNICATION

Acidic Ribosomal Proteins and Histone H3 from *Leishmania* Present a High Rate of Divergence

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Another additional peculiarity in Leishmania *will be discussed about of the amino acid divergence rate of three structural proteins: acidic ribosomal P1 and P2b proteins, and histone H3 by using multiple sequence alignment and dendrograms.*

These structural proteins present a high rate of divergence regarding to their homologous protein in Trypanosoma cruzi. *At this regard,* L. (V.) peruviana *P1 and* T. cruzi *P1 showed 57.4% of divergence rate. Likewise,* L. (V.) braziliensis *histone H3 and acidic ribosomal P2 protein exhibited 31.8% and 41.7% respectively of rate of divergence in comparison with their homologous in* T. cruzi.

Key words: *Leishmania* - acidic ribosomal proteins - histone H3

Acidic ribosomal proteins known as P (phospho) proteins are present in the ribosomes belonging to the eubacteria, archaebacteria and eukaryotes. These conserved proteins are placed in the large ribosomal sub-unit forming a highly flexible lateral projection referred as the ribosomal stalk (Wittman 1983, Lake 1985). The stalk is involved in the interaction between the translation factors and the ribosome during protein synthesis (Shimmin et al. 1989).

Acidic ribosomal P1 protein in trypanosomatids had not been reported in the *Leishmania* genus previous to this work. However, the P0 and P2 ribosomal proteins had been characterised in *L. infantum* (Soto et al. 1995a, b), *L. chagasi* (Skeiky et al. 1994) and *L. donovani* (Kunz et al. 1993). Moreover, *Trypanosoma cruzi* acidic ribosomal protein belong to a gene family found in multiple copies (TcP0, TcP1, TcP2a and TcP2b). The P proteins are important antigens that generate humoral response in leishmaniasis, Chagas disease and systemic lupus erythematosus (Elkon et al. 1986).

Despite of presenting histones, the trypanosomatids do not condense their chromatin during mitosis (Solari 1980). Likewise, trypanosomatids histone H3 presents an extremely divergent N-terminal domain (Galanti et al. 1998). Another important peculiarity is that trypanosomatids histone H3 lacks of the amino acids sequence KSTGGKA at the N-terminal end, which is present in the consensus sequence of higher eukaryotes (Wells 1986).

Three clones from *L. (V.) peruviana* (T26-U3) and *L. (V.) braziliensis* cDNA libraries (T166-U19 and T166-M49) were previously selected by their sero-reactivity with leishmaniasis patients (Montoya 1993). The DNA insert corresponding to each of them was amplified and sub-cloned in pUC18/*Eco*RI/BAP (Pharmacia) plasmid vector.

After sequence analysis, the T26-U3 clone was identified as a *L. (V.) peruviana* acidic ribosomal protein P1 being referred as LpP1. The clone T166-U19 was recognised as a *L. (V.) braziliensis* acidic ribosomal protein P2b and referred as LbP2b. Finally, the T166-M49 clone was identified as a *L. (V.) braziliensis* histone H3 being referred as LbH3.

The deduced amino acid sequence of the LpP1 had a divergence of 57.4% in comparison with *T. cruzi* P1 protein (TcP1). LpP2b showed 17.9% of divergence with the *L. donovani* ribosomal P2 protein (LdP2) and 41.7% of divergence with their

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homologous in *T. cruzi* (TcP2). Finally, LbH3 presented 17.7% and 31.8% of divergence in comparison with the sequences of *L. infantum* (LiH3) and *T. cruzi* (TcH3) respectively (Table).

Multiple alignment of sequences was performed using CLUSTALW 1.5 program and also for establishing the phylogenetic relationships among these proteins was used PHYLIP 3.5c program. For this purpose, eight eukaryotic P2 proteins (Fig. 1) and six histones H3 (Fig. 2) were included. The bootstrap values (bv) were obtained from a consensus tree based on 100 randomly generated trees.

The genetic distances were obtained by Kimura method showing a higher value between LpP1 and TcP1 (0.83902), in contrast with the value observed between LbP2b and TcP2 (0.39811), and also the value obtained between LbH3 and TcH3 (0.38273) (Table). Likewise, LbP2b was very related to *L. donovani* P2 protein whose phylogenetic distance was 0.18489 and their divergence rate was 17.9%. Therefore, our results show that these structural proteins present a high rate of divergence regarding to their homologous protein with *T. cruzi.*

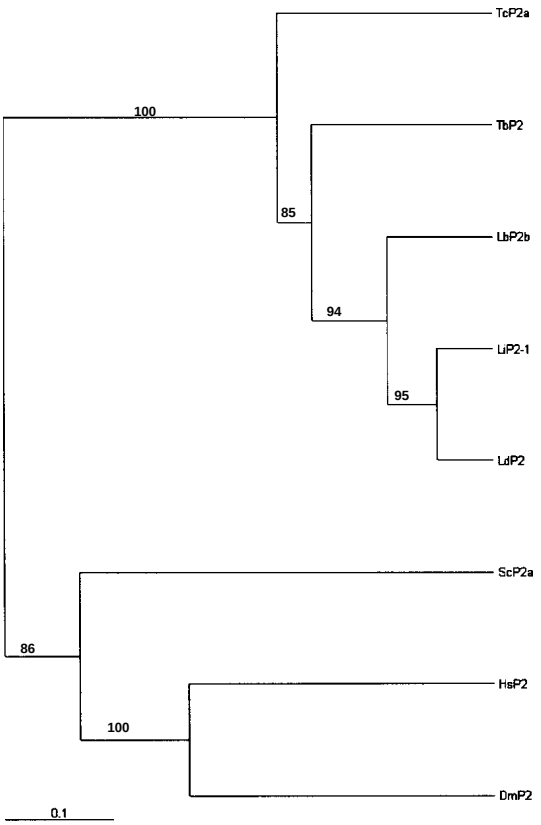

Fig. 1: phylogenetic analysis of P2 acidic ribosomal proteins. Phylogenetic relationships were inferred by using PHYLIP 3.5c. Evolutionary distance matrices, generated by PROTDIST, were determined by using Kimura method. Matrices were used to construct dendrograms using KITSCH program. Bootstrap values were obtained for a consense tree based on 100 randomly generated trees by using SEQBOOT and CONSENSE in the same package, based on the amino acid sequences of 8 P2 protein: *Leishmania brasiliensis* (LbP2b), *L. donovani* (LdP2), *L. infantum* (LiP2-1), *Trypanosoma cruzi* (TcP2a), *T. brucei brucei* (HsP2). Phylogenetic distances scale, is shown in left botton corner. Value indicated in each nodes represent bootstrap value in the respective branch.

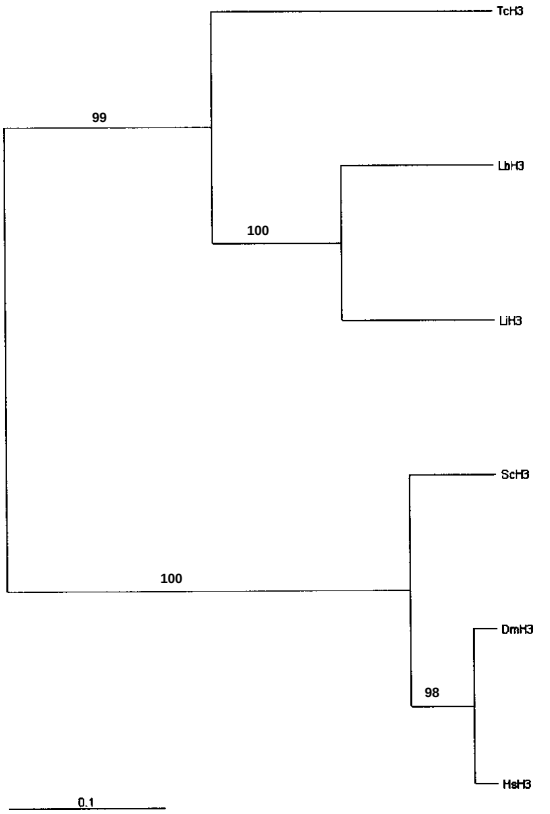

Fig. 2: phylogenetic analysis of histone H3 proteins based on the amino acid sequences of six histone H3 proteins (b): *Leishmania brasiliensis* (LbH3), *L. infantum* (LiH3), *Trypanosoma cruzi* (TcH3), *Saccharomyces cerevisiae* (ScH3) and *Homo sapiens* (HsH3). Phylogenetic distances scale is shown in left botton corner. Value indicated in each nodes represent bootstrap value in the respective branch.

Our results based on the analysis sequence of the *L.(V.) braziliensis* histone H3 and *L. (V.) peruviana* P2 proteins indicate that *Leishmania* is monophyletic. Trypanosomatids P2 clade was separated from the higher eukaryotes clade and sustained with a high bootstrap value (100%) (Fig. 1). In a similar way, trypanosomatids histone H3 clade was separated from the higher eukaryotes clade with a high bootstrap value (99%) (Fig. 2).

Histones H3 are structural proteins considered as one of the most conserved proteins. For example, the divergence rate reported previously between

TABLE

The percentage of amino acid divergence (below diagonal) and phylogenetic distances calculated by Kimura method (above diagonal) among trypanosomatids acidic ribosomal P and histone H3 protein. In parenthesis number of conservatives changes and no conservatives changes

	LpP1	**TcP1**	**ScP1a**	**ScP1b**	**DmP1**	**HsP1**		
LpP1		0.83902	1.05672	1.09255	0.92079	0.93689		
TcP1	**57.4% (20/32)**		0.87003	0.79851	1.01262	0.71531		
ScP1a	62.5% (20/36)	61.5% (15/35)		0.64449	0.87895	0.98490		
ScP1b	63.4% (24/32)	58.1% (16/31)	46.8% (20/25)		1.03140	1.08584		
DmP1	57.9% (24/32)	86.2% (28/31)	57.0% (22/33)	61.4% (28/32)		0.58440		
HsP1	59.8% (21/35)	50.9% (17/33)	61.5% (20/38)	64.1% (24/37)	44.0% (18/27)			
	LbP2b	LdP2	LiP2-1	TbP2	TcP2a	ScP2a	DmP2	HsP2
LbP2b		0.18489	0.20764	0.40351	0.39811	0.73231	0.96413	0.89606
LdP2	**17.9%** (10/7)		0.10209	0.30449	0.36431	0.82614	0.75820	0.86709
LiP2-1	**18.9%** (12/7)	**10.4%** (3/7)		0.32018	0.43335	0.88412	0.88412	0.90973
TbP2	**43.1%** (13/17)	**37.9%** (11/13)	**39.3%** (11/14)		0.41084	0.91716	1.06912	1.03662
TcP2a	**41.7%** (10/20)	**40.0%** (9/19)	**44.0%** (8/24)	**38.9%** (10/22)		1.08439	1.04208	1.18399
ScP2a	55.3% (16/28)	57.2% (18/30)	59.7% (20/30)	62.4% (18/33)	65.2% (21/36)		0.93983	0.67660
DmP2	64.2% (23/29)	58.3% (17/28)	61.7% (18/32)	68.3% (19/37)	64.7% (19/39)	60.3% (22/32)		0.56563
HsP2	60.5% (25/27)	59.7% (21/30)	60.5% (24/29)	64.2% (25/33)	67.5% (25/37)	52.9% (19/26)	48.4% (14/27)	
	LbH3	LiH3	TcH3	ScH3	HsH3			
LbH3		0.19402	0.38273	0.60450	0.60450			
LiH3	**17.7%** (13/9)		0.33693	0.54670	0.57849			
TcH3	**31.8%** (17/22)	**29.5%** (12/23)		0.72004	0.75840			
ScH3	45.3% (15/39)	43.1% (10/39)	49.6% (15/46)		0.10249			
HsH3	42.3% (17/37)	44.5% (12/39)	51.1% (16747)	9.6% (4/9)				

Number accessions of P1 proteins: LpP1, *Leishmania peruviana* (AF045249); TcP1, *Trypanosoma cruzi* (X65025); ScP1a, *Saccharomyces cerevisiae* (PO5318); ScP1b, *S. cerevisiae* (P10622); DmP1, *Drosophila melanogaster* (Y00504) and HsP1, *Homo sapiens* (NM001003). Number accessions of P2 proteins: LbP2b, *L. braziliensis* (AF045020); LdP2, *L. donovani* (O43940); LiP2-1, *L. infantum* (Q06383); TcP2a, *T. cruzi* (P23632); TbP2, *T. brucei brucei* (P51408); ScP2a, *S. cerevisiae* (PO5319) and HsP2, *H. sapiens* (NM001004). Number accessions of histone H3: LbH3, *L. braziliensis* (AF044682); LiH3, *L. infantum* (P40285); TcH3, *T. cruzi* (AAA61706); ScH3, *S. cerevisiae* (P02303) and HsH3, *H. sapiens* (NP 005315).

human and *Saccharomyces* histone H3 was just 9.6% (Table). However, in *Leishmania* a high rate of divergence at the N-terminal end and the separation of the trypanosomatids clade out of the higher eukaryotes clade is reported here (Fig. 2). This changes in the N-terminal end could contribute in the lost of chromatin condensation.

The divergence rate observed between P1 proteins (LpP1 and TcP1 57.4%) showed a value fluctuating in the range of the higher eukaryotes (44% to 64.1%) (Table). The low number of sequences reported to this protein did not allow establish the phylogenetic relationship among these proteins.

Further studies should be performed to relate the high divergence rate detected in these parasites with their functional implications during the translation.

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