Upstream open reading frames (uORFs) in *Trypanosoma cruzi*: search of peptides that might regulate translation of proteins.

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uORFs are short sequences of nucleotides that have a start codon and are present in regions considered to be untranslated of mRNA. These sequences have been described in mRNAs from several organisms. Activities include regulation of stability, inhibition of translation and cis regulation, both positive and negative. Due to their small size and specific activities, the detection of peptides coded from uORFs has required indirect methods; assays of *in vitro* translation, gene reporter systems or bioinformatics analysis. In this work, we used a method to directly detect and identify small peptides coded by uORFs in *T. cruzi*, through a mass spectrometry-based methodology combined with 1D SDS-PAGE, genomic mapping, and computational analysis of the peptide sequence.

Protein extracts from epimastigotes of *T. cruzi* (CL Brener strain) were obtained using two methodologies: lysis by freeze/thaw cycles, followed by centrifugation, or by using Tryzol® reagent. Proteins in the extract were separated by 15% SDS-PAGE and silver stained. Regions below 20 kDa were excised from the gel, digested and resulting peptides were purified by ZIP-TIP-C_{18}®. Samples were analyzed by MALDI-TOF/TOF mass spectrometer. Peptide sequences were obtained and identified by Mascot® program, then compared with the *T. cruzi* genome by TBLASTN in NCBI database.

We identified 9 proteins using both methods. Trizol extraction showed to be more effective. All identified proteins were found in the open reading frame regions of the genome. None of them corresponded to the small peptides from the uORFs. It is likely that these peptides are presented in very low amounts or they have a short half-life in the cell. We are now working on more sensitive separation and purification methods to detect these peptides.

Key-words: uORFs, mass spectrometry, *Trypanosoma cruzi*.
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