Trypanosoma cruzi: CLONING OF THE 26 S RIBOSOMAL GENE AND ITS ASSOCIATED SPACER.

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The ribosomal RNA species (rRNAs) of *Trypanosoma cruzi* differ considerably from those of other eukaryotes studied to date. The rRNA of the large subunit (26 S) is cleaved yielding two smaller rRNAs of 16 S and 18 S, respectively (1,2,3,4). The small subunit contains a single rRNA of 20 S. A partial map of the *T. cruzi* ribosomal repeating unit was constructed based on genomic Southern blotting hybridizations using labeled *T. cruzi* rRNAs as probes (5).

The structural analysis of ribosomal genes and their associated spacers, has emerged as an important tool for taxonomic and phylogenic studies. Recently, a cloned ribosomal spacer of *Leishmania braziliensis* has been used in the identification of *Leishmania* species (6). Hereby we describe the isolation and characterization of a *T. cruzi* genomic fragment that carries part of the 18 S coding sequence, the whole coding sequence for the 16 S rRNA and a large portion of the outer ribosomal spacer. Further, we used this recombinant clone in a comparative analysis of ribosomal genes in different *T. cruzi* strains and trypanosomatids.

In order to isolate ribosomal genes, a *T. cruzi* genomic library was subjected to a differential screening using two distinct probes: *T. cruzi* rRNAs labeled with ^{32}P by

Polynucleotide Kinase and a cloned fragment containing part of 3' end of 26 S rRNA gene of *Leishmania braziliensis* (6). This differential screening allowed us to isolate recombinant clones carrying specific sequences of *T. cruzi* rDNA gene cluster. We have isolated clones that contain sequences for the 26 S coding region and clones encoding the 20 S rRNA.

Clone 4al is a recombinant phage containing a large portion of the *T. cruzi* ribosomal repeating unit. The 14.7 Kb long clone includes part of the 18 S coding sequence, the whole coding sequence for the 16 S rRNA and the 3' end outer ribosomal spacer. Southern blot analysis show that clone 4al hybridizes to several *T. cruzi* genomic fragments suggesting the presence of repetitive sequences probably localized within the cloned spacer region. When this analysis was extended to different *T. cruzi* strains, specific patterns for each strain were observed. These results suggest that *T. cruzi* outer ribosomal spacer sequences can discriminate between strains.

Similar analysis for other trypanosomatidae species, showed a restricted hybridization pattern, that we ascribed to the conserved transcribable region within clone 4al. In spite of the sequence homology of the rRNA coding region among different trypanosomatids, we observed a restriction lenght polymorphism. This result, if standardized, could be useful as a taxonomical criterion.

The data presented here reinforce the evidences that the study of rRNA cluster in different members of trypanosomatid family could be used as an additional tool among new taxonomic parameters within this group, as well as to compare different *T. cruzi* strains.

References:

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