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ISOLATION AND CHARACTERIZATION OF POLYMORPHIC MICROSATELLITES FROM THE CL BRENER CLONE OF *Trypanosoma cruzi*

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Microsatellites are short tandem repeat sequences dispersed within eukaryotic genomes. These sequences frequently exhibit length polymorphism constituting extremely informative marks for genetic analysis in a great variety of organisms. In this work, we investigate the occurrence of microsatellites in the genome of CL Brener clone of Trypanosoma cruzi to be used as markers for genetic mapping. Although no T. cruzi microsatellites were reported until now, the searching for di- and tri-nucleotide motifs using the GCG Findpattern program in T. cruzi Genbank sequences showed that the most frequent kind of microsatellites are AC and AG stretches with a minimum of 6 and maximum of 15 repeats in length. Hybridization analysis using alkaline phosphatase labeled $(CA)_n$ probe on the molecular karyotype of CL Brener clone showed the presence of this microsatellite in all chromosomal bands. To characterize microsatellite loci, a genome library enriched with CA repeats was constructed. For that, genomic DNA was digested with MseI or Sau 3AI and ligated to adaptors. Denatured restriction fragments were hybridized to a biotinylated probe complementary to CA repeats and immobilized on a streptavidin coated magnetic beads. The captured fragments were submitted to a PCR reaction using primers complementary to the adaptors and then cloned in a TA-vector. More than 90% of the recombinant clones hybridized to the $(CA)_n$ probe. Sequencing of 25 positive randomly selected clones showed that all contained a CA repeat length greater than four and 60% of these clones contained a CA repeat greater than nine. Several clones showed association with other microsatellites such as (A)n, (AG)n, (AAC)n and (AATT)n. Fluorescein-labeled primers were designed to amplify the CA repeat loci and products were studied in the ALF sequencer using the Fragment Management program. Five clones displayed allelic size polymorphism among different *T. cruzi* strains. Localization of these clones on the molecular karyotype of the CL Brener clone is being performed. Isolation and analysis of more microsatellites loci will be useful for future work in genetic mapping of *T. cruzi* as well in population studies.
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GENETIC STRUCTURE AND EVOLUTION OF *Trypanosoma cruzi*, THE AGENT OF CHAGAS DISEASE, AS REVEALED BY MULTIPRIMER RAPD ANALYSIS: CLONALITY VS. SEXUALITY

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Trypanosoma cruzi natural populations have been shown to be highly heterogeneous and subdivided into a number of genetically distant clonal lineages. We have selected 21 highly resolutive primers, out of 120 tested, to analyse 50 stocks representative of the major genetic subdivisions of the parasite. Stocks were isolated from diverse hosts and from different geographic areas. Other trypanosomatids, including *Leishmania* and *T. congolense*, were analysed in parallel in order to directly compare their genetic diversity with *T. cruzi*.
The results show the marked genetic structuration of *T. cruzi* in a few distinct genetic groups, confirming previous MLEE and RAPD data. These groups are interpreted as clonal lineages. RAPD markers specific for each group are numerous and will be used to develop probes for specific diagnostic purposes.
The huge genetic heterogeneity of *T. cruzi* is confirmed by the divergence between lineages of this parasite which is similar to that observed between species, and even species complexes, within the *Leishmania* genus.
The phylogenetic relationships between the *T. cruzi* groups were investigated by multivariate and phylogenetic analysis. The monophyly of each group appears robust, but the hierarchical relationships between groups are problematic. Moreover, some groups display features that could be interpreted as the result of a hybridization event between strains of two other groups.
These findings suggest the possibility of an evolutionary process involving both occasional recombination leading to new genotypes and clonal evolution of successful lineages.