Genome wide transcript profiling of kinetoplastids

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Comparative genomics, i.e. comparison of whole genome sequences of different organisms, is becoming feasible due to the increasing availability of complete sequences. Genome wide analysis is a powerful way to study the biology of closely related organisms by looking for instance at differential gene organization and expression, sequence structural organization, etc., providing the foundation for understanding how living cells function and live, and how they interact with their environments and with other organisms. While organisms with their genome completely sequenced might be so analyzed, it is still not possible for the vast majority for which no complete sequence is available. Especially with kinetoplastids, protozoa of the order Kinetoplastida, family Trypanosomatidae, with a wide range of medical, agricultural and veterinary importance as well as with no importance at all from these points of view, most of its member's genomes will be sequenced far in the future, if ever, given the present sequencing technology. Due to this shortcoming, we are testing an alternate genome-wide survey, of coding sequences first, of the main representatives of kinetoplastids, using PCR amplification assay and primers based on EST's of one kinetoplastid, Trypanosoma cruzi. We will initially use the set of primer-pairs we presently have which were designed for making a T. cruzi DNA spotted microarray. This is a collection of approximately four thousand (4,000) primer-pairs based on 9,919 T. cruzi EST's retrieved from EMBL database Release 62.0 and other sequences deposited in GenBank. We estimate that T. cruzi has 6-8,000 genes. We are in the process of obtaining the remaining EST's by sequencing normalized libraries of cDNA prepared from mRNA of the parasite at different stages of differentiation. We will also use our available T. cruzi microarray for these surveys by directly hybridizing labeled genomic DNA from other kinetoplastids.