Searching for signals in the Tritryps genomes

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The protozoans \textit{Trypanosoma cruzi}, \textit{Trypanosoma brucei} and \textit{Leishmania major} (Tritryps), are evolutionarily ancient eukaryotes which cause worldwide human parasitosis. Probably due to the early branching in eukaryotic evolution, they present unique biological features. Particularly, RNA polymerases (RNAP) are distinctive. The search for transcription initiation sites has been elusive so far. Polycistrons strand switch regions have been implicated in this process. Besides, \textit{Trypanosoma brucei} ARNPI is capable of synthesizing the pre-mRNA\textsubscript{m} coding for VSG and procyclin proteins. In eukaryotes, RNAPI promoters are characterized by the conservation of conformational elements but no sequence conservation.

We aim to determine the structural characteristics of transcription initiation sites in Tritryps and establish a prediction system for putative binding sites. Apart from this, we plan to carry out an experimental approach including the characterization of each polymerase transcriptome. In this regard, we have collected the sequences corresponding to the RNAPI transcription start sites of about 25 eukaryotes (including the ones for Tritryps). We have also collected the sequences corresponding to all the strand switch regions of Tritryps. With these sequences we constructed databases of their conformational characteristics. The results are being used to establish a predictive system that we hope will classify regions of the genome of the parasites as potential binding sites for these polymerases.

Experimentally, we are carrying out run-on assays in the presence of \(\alpha\)-amanitin to establish the transcriptome of RNAPI. We conducted these experiments with \textit{T. brucei} procyclic forms. qRT-PCR analysis of the sub-population of RNAs obtained established that the methodology is able to separate RNAs synthesized \textit{de novo} but the conditions for RNAPI inhibition should be adjusted.