The interplay between chromatin conformation, epigenetics and global transcription in *Trypanosoma cruzi*.

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In trypanosomes, the organization of genes and their regulation exhibit distinct features compared to other eukaryotes. They are arranged into directional gene clusters (DGCs) interspersed with strand-switch regions, where transcription directionality converges or diverges. These DGCs are transcribed as polycistronic transcriptional units (PTUs), co- or post-transcriptionally processed. Post-transcriptional regulation is widely identified as the primary mechanism of gene expression control in these parasites. In recent years, some findings have underscored the significance of epigenetics and chromatin organization in governing gene expression, cell cycle regulation, and differentiation.

The genome of *T. cruzi* is divided into core and disruptive compartments. The core is syntenic with *T. brucei* and *Leishmania spp.*, while the disruptive (disruption of synteny) comprises hundreds of MASP, TS and TcMUC surface proteins coding genes. This arrangement closely resembles that of *T. brucei*, except that in these parasites, the disruption of synteny occurs in the subtelomeric regions, where the VSGs are present. We can then establish that in trypanosomes, the disruptive compartment is species-specific, and their genes are directly related to the infection in the mammal host. This genomic organization in *T. cruzi* led us to hypothesize a correlation with chromatin conformation, directly impacting gene expression.

By analysing chromatin conformation and global transcriptomics across various stages of *T. cruzi*, we have observed a correlation between one-dimensional and three-dimensional organization of the genome. Analogous to compartments A and B in some eukaryotes, trypanosomes exhibit C and D (core-related and disruptive-related, respectively), with C being less densely packed. Moreover, chromatin is organized into chromatin folding domains (CFDs) that differ in size and frequency of interaction between the compartments. When the contacts between chromosomes were analyzed, most were intra-chromosomal in C, while in D, both intra and inter-chromosomal interactions were found.

Transcriptomics analysis showed that transcription start and end regions do not coincide with the DGCs, but with the CFD, challenging the widely accepted DGC-PTU correspondence and demonstrating that transcription is significantly affected by the local chromatin structure. The same correlation was observed in *T. brucei*. In the C compartment most of the genes are expressed throughout the life cycle, while the D compartment functions as a specialized region primarily active during the infective stages. These findings challenge the conventional understanding that these parasites transcribe genes indiscriminately, followed by post-transcriptional modulation of their expression. While this pattern is observed in the C compartment, it does not hold for the D compartment, where only a small fraction of the numerous genes in each multigenic family are highly expressed. In both *T. cruzi* and *T. brucei*, the D compartment comprises regions with high chromatin compaction, potentially facilitating gene-to-gene regulation, preventing spurious transcription, and promoting intra- and inter-chromosomal recombination.

Finally, compartments C and D are differently susceptible to epigenetic changes. The analysis of nucleosome positioning data in relation to C and D showed that well-positioned nucleosomes abound in D. On the other hand, the study of DNA methylation marks demonstrated that 6mA is present at low levels while 5mC is an abundant modification and is predominant in the core compartment.

For the *T. cruzi* D compartment, we propose a model where stochastic events of compaction and recombination generate antigenic diversity through the expression of a discrete number of surface proteins that allow parasites to evade the immune response.