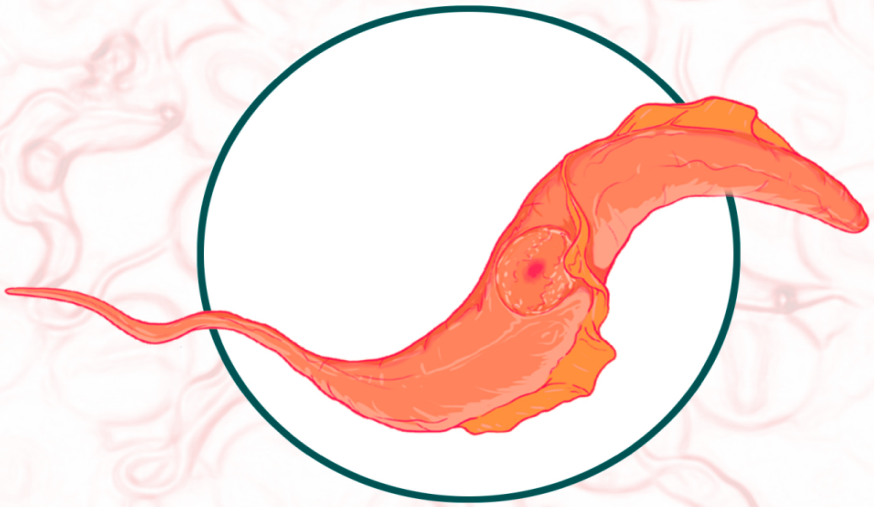

ABSTRACTS OF MAIN LECTURES
IX SYMPOSIUM ON MOLECULAR BIOLOGY OF CHAGAS DISEASE

RESÚMENES CONFERENCIAS MAGISTRALES
IX SIMPOSIO DE BIOLOGÍA MOLECULAR DE LA ENFERMEDAD DE CHAGAS

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Diagnostic of Chagas Disease in Mexico

Diagnóstico de la Enfermedad de Chagas en México

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There are 8 million people infected with the *Trypanosoma cruzi* parasite worldwide, and over 10,000 die every year. Chagas disease is found mainly in the Latin American region, although it has been reported in 17 European countries and in the Western Pacific region (due to migration). Urbanization processes in Latin America during the 20th Century and migration have changed the epidemiology of the disease, turning it into a global risk. The Health Office Administration in Mexico (in Spanish: Secretaría de Salud) proposed to control the vectorial transmission through risk stratification, housing development, chemical vector control, and elimination of connatal and transmission through transfusion of Chagas. The National Institute of Epidemiological Reference (in Spanish: INDRE) processed 33,277 samples from 2001 to 2017; 15,878 (48 %) were positive for Chagas disease. In 2018, the states with the highest rate of incidences were Yucatan (0.8), Quintana Roo (0.8) and Nayarit (0.7). The most crucial vectors identified in some states of the country were: *Triatoma dimidiata*, followed by *T. mexicana* and *T. gerstaeckeri*; TcI is the biotype with which they are mainly infected. There are few studies about the lineage of TcII-TcVII in Mexico, and in these studies appear the presence of several *T. cruzi* lineages on feces from several vectorial species (*T. dimidiata, T. longipennis* y *Meccus pallidipennis*) across Mexico. In Nuevo León, the only current identified vector was *T. gerstaeckeri*; in 2014 the seroprevalence of *T. cruzi* antibodies was 1.93 % (52/2,688); in the electrocardiogram study, 22.85 % (8/35) of infected individuals presented abnormalities in the ECG. These relevant findings in the northeaster region of Mexico place Chagas disease as a serious danger for public health. As for the diagnosis in Mexico, INDRE mentions conventional techniques like parasitological, antibodies detection like immunofluorescence, hemagglutination (HIA) and immunosorbent essay linked to enzymes (ELISA). For pregnant women in blood samples, the molecular diagnosis by polymerase chain reaction (PCR), was the confirmatory method in cases with patients with megaoesophagus, whom has doubtful serology, and bank bloods, while this technique is expensive to be used as a routine test, it can be very helpful in those cases where the infection of the parasites is recent, and the antibodies presence is yet to be detectable. For discrete typification of units (DTU) identification of *T. cruzi* in biological samples, real-time multiplex PCT (MTq-PCR) is the most useful: as well as the intergenetic region (SL-IR), 18S, 18S-AND ribosomal; cytochrome oxidase II (COII); 24Sα, 24Sα-ADN ribosomal; MTq multiplex TaqMan. Entomological work consolidation, such as vectorial control, improvement in housing development, screening of pregnant women living in a risk zone, and universal screening of blood and organ donors, will make possible the elimination of congenital and transfusion transmission.

Keywords: Chagas, diagnosis, prevalence, Mexico, Nuevo Leon

Palabras clave: Chagas, diagnóstico, prevalencia, México, Nuevo León

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Tc323, a *T. cruzi* novel protein with diagnostic potential

Tc323, una nueva proteína de *T. cruzi* con potencial diagnóstico

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Chagas illness, a potentially life-threatening disease, is an infection caused by the hemoflagellate parasite *Trypanosoma cruzi*. Since nowadays none of the available diagnostic methods disclose ∼100% specificity and sensitivity during chronic phase of Chagas disease (CCD), the WHO advises the use of at least two of distinct serological tests for the reliable diagnosis of CCD. From this perspective, we evaluated the diagnostic utility of a hypothetical protein *T. cruzi*, called Tc323, which is only present in this parasite. This protein was identified as the target of a single-chain variable fragment recombinant antibody (scFv 6B6) isolated from a phage display library constructed from B cells of chronic Chagas heart disease patients. Phylogenetic analysis showed that Tc323 is highly conserved throughout evolution in all *T. cruzi* lineage but it lacks orthologous in other kinetoplastid parasites. Due to experimental limitations to produce the full-length protein as recombinant, structural predictions using RaptorX server allowed us to foretell six structural domains (D1-D6) which were cloned into pRSET-A vector and expressed in *E. coli* BL21 Rosetta (DE3) cells. In addition, B cell epitopes predictors mapped that almost all the protein is immunogenic, while the majority of antigenic sequences are within D3 and D6 domains. After optimization of direct ELISA by checkerboard titration, preliminary results showed that recombinant His-tag D3 and D6 domains were recognized by antibodies present in plasma from individuals with CCD but not from those coming from patients with *Leishmania* and non-infected donors. Our finding allowed the identification of two recombinant domains containing Tc323 as a promising immunodiagnosis candidates for chronic Chagas disease in human.

Keywords: chronic Chagas disease, *T. cruzi*, serologic diagnostic, hypothetical proteins

Palabras clave: enfermedad de Chagas crónica, *T. cruzi,* diagnóstico serológico, proteínas hipotéticas

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Point-of-care molecular diagnosis of congenital Chagas disease: from the laboratory to the Maternity

Diagnóstico Molecular de Chagas congénito en puntos de atención: Del laboratorio a la maternidad

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Mother-fetal transmission of *T. cruzi*, causing **congenital Chagas disease (cCD)**, represents one of the main global scenarios of CD, involved in urbanization of CD in endemic and non-endemic countries. Enhancing access to early cCD diagnosis should be a priority at national and regional levels because its prompt treatment achieves a high cure rate, precluding evolution to chronic CD. However, current algorithms involve low sensitive parasitological assays, making necessary serological confirmation after nine months of life. Mainly due to

economical constrains, a high proportion of infants, especially from rural areas is lost to follow-up. Molecular methods for early diagnosis in neonates have been tested to bypass this loss. A TaqMan real-time PCR kit has achieved higher sensitivity than micromethod starting from 1 mL of blood. Studies in infants born to seropositive mothers observed that the first month of life is the best opportunity to perform PCR, when the parasitic load is at its peak and potential false positive results that might arise from contamination with maternal *T. cruzi* DNA are minimised. Loop mediated isothermal amplification (LAMP), is an alternative molecular approach, suitable to resource-limited laboratories, because it does not require a thermocycler, but only a thermoblock or water bath. Furthermore, product visualisation can be done by the naked eye or fluorescence. A prototype kit based on the parasite satellite DNA, that contains dried reagents on the inside of the microtube caps (Eiken Chemical Co, Japan) has been evaluated in archival panels of cCD samples. Aiming at implementing early diagnosis in minimally equipped laboratories associated with Maternities in endemic countries, this *T. cruzi*-LAMP kit was coupled to different rapid DNA extraction methods and supports: 1. An automated DNA extraction device re-purposed from a 3D printer (PrintrLab extraction device, AI Biosciences Inc., College Station, Texas, USA) that uses a Multi Sample DNA extraction kit based on magnetic beads. It has been optimized for 100-200 μL of blood anticoagulated with EDTA and takes less than three hours to yield a result. A recent pilot study in Yacuiba and Villa Montes, Bolivia, showed promising findings with higher sensitivity than the micromethod and high agreement with PCR. 2. An ultra-rapid DNA extraction method (PURE, Procedure for Ultra Rapid Extraction-Eiken Chemical Co, Japan) that uses only 30 μL of starting blood anticoagulated with heparin and the DNA is obtained in around 10-15 minutes. This approach has been analytically validated in blood samples containing serial dilutions of cultured parasites of different discrete typing units and is under prospective evaluation in a multicentre POC study. The more recent challenge is to test dried blood spots using Flinders Technology Associates (FTA®) cards to expand LAMP diagnosis for babies born in domiciles or rural areas without a laboratory suitable for LAMP procedures. These procedures are being actually transferred to laboratories linked to Maternities in endemic sites of Argentina, Bolivia and Paraguay.

Financial Support: ChagasLAMP Project (ref.: G2020-203) from the Global Health Innovative Technology Fund and Small grant program of PAHO/WHO/TDR (LEG ID39002).

Keywords: *Trypanosoma cruzi*, congenital Chagas disease, molecular diagnosis, polymerase chain reaction, loop mediated isothermal amplification, Real time-PCR, LAMP

Palabras clave: *Trypanosoma cruzi*, Chagas congénito, diagnóstico molecular, Reacción en cadena de la polimerasa, amplificación isotérmica mediada por asas, PCR en tiempo real, LAMP

Host-*Trypanosoma cruzi* interactions: role of placenta-specific micro-RNAs during congenital transmission

Interaccción hospedero-*Trypanosoma cruzi*: rol de micro-ARNs placenta-específicos en la transmisión congénita

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Congenital Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is responsible for 22.5% of

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new cases yearly. However, placental transmission occurs only in 5% of infected mothers, and it has been proposed that the epithelial turnover of the trophoblast is considered a local placental defense against the parasite. Thus, *Trypanosoma cruzi* induces cellular proliferation, differentiation, and apoptotic cell death in the trophoblast, which are regulated by small non-coding RNAs such as microRNAs. On the other hand, *ex vivo* infection of human placental explants induces a specific microRNA profile that includes microRNA related to trophoblast differentiation and apoptotic cell death, such as miR-512-3p miR-515-5p codified at chromosome 19 microRNA cluster (C19MC). Here we determined the expression of miR-512-3p and miR-515-5p validated target genes, specifically human glial cells missing 1 transcription factor and cellular FLICE-like inhibitory protein. In addition, we evaluated the expression of the main trophoblast differentiation marker human chorionic gonadotrophin and caspase 8 during *ex vivo* infection of human placental explants. Moreover, we analyzed how inhibition or overexpression of both microRNAs affects parasite infection. We conclude that the *Trypanosoma cruzi*-induced trophoblast epithelial turnover, particularly the trophoblast differentiation, is at least partially mediated by the placenta-specific miR-512-3p and miR-515-5p and that both miRNAs mediate placental susceptibility to *ex vivo* infection of human placental explants. Knowledge about the role of parasite-modulated microRNAs in the placenta might allow, in the future, their use as biomarkers, prognostic and therapeutic tools for congenital Chagas disease.

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Keywords: Chagas Disease, micro-RNAs, congenital transmission, placenta

Palabras clave: enfermedad de Chagas, micro-ARNs, transmisión congénita, placenta

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What are the pathways in the DNA damage response: growth arrest, dormancy or death?

¿Cuáles son las vías de respuesta al daño del ADN: detención del crecimiento, latencia o muerte?

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Trypanosoma cruzi is the etiological agent of Chagas disease, a neglected illness that affects millions of people. Thereby, understanding how this pathogen survives stressful conditions can provide insights into its biology and discovery of new drugs. Exposing *T. cruzi* to agents that cause DNA lesions is a stressful condition that can compromise its survival. In this context, a network of pathways called the DNA damage response (DDR) coordinates the repair of lesions to maintain the integrity of the genome. If the damage is not repaired properly, DDR proteins can initiate a signaling cascade for arrested the cell, dormancy or programmed cell death. Of the factors that cause DNA damage, UV and gamma radiation generates lesions that can block or arrest RNA polymerase or DNA polymerase. Such disruption can have a highly deleterious effect on *T. cruzi*, as it transcribes several genes into a single polycistronic pre-mRNA. In this parasite, UV radiation causes immediate apoptosis-like cell death dependent on ATR signaling, a DDR key protein kinase. Interestingly, *T. cruzi* does not repair nuclear UV lesions up to 24h. However, it does not demonstrate increased sensitivity to UV radiation until

this time, suggesting that UV lesions per se are not directly involved in directing cell death. Thus, it is likely that other factors may modulate this signaling process. Blocking RNA polymerase can give rise to R-loops, a structure that can form when RNA hybridizes with DNA, generating a DNA/RNA hybrid and displaced single-stranded DNA. Although R-loops perform physiological functions, they are also associated with genomic instability and could be important to signal to the death. Unlike what happens with UV-induced lesions, lesions caused by gamma radiation cause double strand breaks (DSBs) which block the replication process. This type of injury leads to cell cycle arrest but is not capable of leading to signaled cell death. One of the main markers of the presence of DSBs is the phosphorylated gamma histone 2A (pH2Ax), and the analysis of this modified histone shows that after gamma irradiation there is a large increase in their presence, which may be associated with cell cycle arrest due to the DDR and the action of the ATM protein. Interestingly, even after cell growth resumes, it is possible to verify that pH2Ax levels do not return to pre-irradiation levels. We are investigating whether the maintenance of phosphorylated histone may be related to the presence of dormant cells that increase after gamma irradiation. With these data, our hypothesis is that depending on the triggered DDR we can have different processes occurring in the parasite. Inhibition of transcription can lead to the formation of R-loops that will activate the ATR protein that may signal cell death. On the other hand, the DSBs will activate the ATM protein which may lead, at first, to the arrest of the cell cycle that will allow the repair of the lesions. However, if the injuries are not properly repaired, the signal will not be turned off and may lead the cell to dormancy.

Financial Support: CAPES, CNPq.

Keywords: *Trypanosoma cruzi*, DNA damage, pathways

Palabras clave: *Trypanosoma cruzi*, daño del ADN, vías de respuesta

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Drug target validation against *Trypanosoma cruzi* through Metabolic Control Analysis and Metabolic Modeling

Validación de blancos terapéuticos contra *Trypanosoma cruzi* mediante el análisis del control y modelado metabólicos

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In the validation of therapeutic targets on the intermediary metabolism of protozoan parasites (and in general of pathological cells to determine the essentiality of a protein) the gene expression silencing method is commonly applied. The results of these analyses generally conclude that ALL silenced metabolic enzymes are essential for parasite survival or infectivity. Therefore, it is necessary to apply additional strategies to identify those enzymes that, in addition to being essential, when inhibited at a lower percentage than those obtained by genetic or pharmacological methods, have negative effects on the parameters of antiparasiticidal activity being sought. In the study of the mechanisms that regulate and control the metabolic pathways of a cell, Metabolic Control Analysis (MCA) and kinetic modeling (a Systems Biology approach that consists of building computational models of cell metabolism) are strategies that allow us to quantify the degree of control that an enzyme has over the fluxes of the metabolic pathway to which it belongs. By means of these strategies it is possible to identify those enzymes that have the greatest control of the pathway, which from the metabolic point of view are the sites with the greatest therapeutic potential. These strategies were applied to trypanothione-dependent

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antioxidant metabolism and cysteine metabolism in the *Trypanosoma cruzi* parasite. *In silico, in vitro* and *in vivo* results indicate that mild/moderate inhibition of enzymes/transporters that have the greatest degree of control of a metabolic pathway have a greater negative effect on the parasite than inhibition of an enzyme with little control. Therefore, MCA and computational modeling of metabolic pathways are strategies to help prioritize sites for therapeutic intervention. These approaches can direct basic research for drug design or drug repositioning against validated therapeutic targets.

Financial support: CONACyT 282663

Keywords: *Trypanosoma cruzi*, trypanothione, antioxidant system, metabolic modeling, COPASI, metabolic control analysis

Palabras clave: *Trypanosoma cruzi*, tripanotión, sistema antioxidante, modelado metabólico, COPASI, análisis del control metabólico

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The role of ESCRT in endocytosis and secretion of extracellular vesicles by *Trypanosoma cruzi*

El papel del ESCRT en la endocitosis y la secreción de vesículas extracelulares por *Trypanosoma cruzi*

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The ESCRT machinery (Endosomal Sorting Complex Required for Transport), consists of four multi-subunit complexes, ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, that play a role in the transport of ubiquitinated cargoes to intraluminal vesicles that bud from multivesicular bodies derived of endosomal compartments. These multivesicular bodies later fuse with lysosomes for degradation of the endocytosed cargoes or with the plasma membrane secreting intraluminal vesicles as a heterogeneous mixture of exosomes. Because Trypanosomatids, which are protozoan parasites that secrete extracellular vesicles and depends on endocytosis, here we analyzed the role of ESCRT complex in *Trypanosoma cruzi*, the agent of Chagas disease. Parasites expressing the Cas9 and T7 RNA polymerase were transfected with DNA fragments coding for two sgRNA that pair the VPS23 gene, a member of the ESCRT-I, together with DNA donor fragments to replace the entire gene by hygromycin and blasticidin resistance markers. Only one of the VPS23 alleles were replaced by one copy of the resistance markers, suggesting that VPS23 is essential for *T. cruzi*. Similar results were obtained for *Leishmania mexicana*. These partial knockouts significantly lower the receptor mediated endocytosis of transferrin, but not the fluid phase BSA-uptake in *T. cruzi*. In addition, they reduced the secretion of extracellular vesicles by epimastigotes. The partial knockout did not affect epimastigote proliferation and metacyclogenesis, and the resulting trypomastigotes were able to infect cells as the parental cell lines. We are currently analyzing whether the secretion of extracellular vesicles is also changed in trypomastigotes released from infected mammalian cells and the capacity of these parasites to promote infection. These results demonstrated the essential role of TcVPS23 by modulating endocytic and secretory activities in *T. cruzi*, and might be useful to understand the role of extracellular vesicles, know to play an important role in the parasite virulence.

Keywords: *Trypanosoma cruzi*, TcVPS23, endocytosis, secretion, vesicles

Palabras clave: *Trypanosoma cruzi*, TcVPS23, endocitosis, secreción, vesículas

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Epigenetics, phenotypic changes, and gene expression in *Trypanosoma cruzi*: a puzzle to be solved

Epigenética, cambios fenotípicos y expresión génica en *Trypanosoma cruzi*: un rompecabezas por resolver

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Genomic organization and gene expression regulation in trypanosomes are remarkable because protein-coding genes are organized into codirectional gene clusters with unrelated functions. Moreover, there is no dedicated promoter for each gene, resulting in polycistronic gene transcription, with posttranscriptional control playing a major role. Nonetheless, these parasites harbor epigenetic modifications at critical regulatory genome features that dynamically change among parasite stages. Here, we will discuss the impact of chromatin changes in a scenario commanded by posttranscriptional control exploring the parasite *Trypanosoma cruzi* and its differentiation program using epigenomic (FAIRE-seq, MNase-seq, Chip-seq and Hi-C data) and transcriptomic (mature and nascent RNA-seq) approaches. The integration of FAIRE and MNase-seq data, two complementary epigenomic approaches, enabled us to identify differences in *T. cruzi* genome compartments, putative transcriptional start regions, and virulence factors. In addition, we also detected a developmental chromatin regulation at tRNA loci (tDNA), which seems to be linked to the translation regulatory mechanism required for parasite differentiation. We are currently mapping the exact moment where the tDNA loci close during metacyclogenesis and its association with tRNAs expression. tDNA locus is also enriched in the histone H2B variant (H2B.V). This latter is also mainly located at dSSSrs and between the core and disruptive genome compartments. Strikingly, a positive correlation was observed between active chromatin and steady-state and nascent transcription levels. Taken together, our results indicate that chromatin changes reflect the unusual gene expression regulation of trypanosomes and the differences among parasite developmental stages, even in the context of a lack of canonical transcriptional control of protein-coding genes.

Supported by: FAPESP, Serrapilheira and CAPES.

Keywords: *Trypanosoma cruzi*, epigenetic, phenotypic changes, gene expression

Palabras clave: *Trypanosoma cruzi*, epigenética, cambios fenotípicos, expresión génica

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Uncovering kinetoplastid-specific components of a super-interactome supporting mRNA export in trypanosomes

Descubrimiento de componentes específicos de cinetoplástidos de un súper interactoma que apoya la exportación de ARNm en tripanosomas

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The nucleocytoplasmic RNA export is an essential pathway for gene expression regulation in eukaryotic cells, but it is still poorly understood in protozoan parasites. In trypanosomatids, transcription is polycistronic and all mRNAs are processed by *trans*-splicing, with export mediated by noncanonical mechanisms. Few orthologs of proteins involved in mRNA export in higher eukaryotes are detectable in trypanosome genomes. We previously described two conserved components of the mRNA export pathway in *T. cruzi*: orthologs of Sub2, a component of the TREX complex, and eIF4AIII (previously Hel45), a core component of the exon junction complex (EJC). Then, we searched for protein interactors of both proteins using cryomilling and mass spectrometry. Significant overlap between TcSub2 and TceIF4AIII-interacting protein cohorts suggests that both proteins associate with similar machinery. The analyses of the TcSub2 and eIF4AIII interactomes identified several interactions with conserved core components of the EJC and uncovered proteins specific to trypanosomatids, named as TcFOP, TcAPI5, TcNTF2L and TcHYP. Additional immunoisolations of the kinetoplastid-specific proteins both validated and extended the superinteractome, showing the presence of proteins involved in the processing, export, quality control and translation of mRNA. Through immunoprecipitation assays coupled with mass spectrometry, we uncover the high connectivity between multiple aspects of mRNA metabolism and kinetoplastid-specific components that create a unique amalgam to support trypanosome mRNA maturation.

Keywords: mRNA export, proteomics, gene expression, trypanosomes

Palabras clave: exportación del ARNm, proteómica, expresión génica, tripanosomas

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Alba-domain protein family of *Trypanosoma cruzi*: An approach to its functions

Aproximación a la función de la familia de proteínas Alba en *Trypanosoma cruzi*

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Trypanosoma cruzi is a parasite characterized by a high genetic diversity and a complex biological cycle that

alternates between two different hosts in which it is subjected to multiple cellular stresses such as thermal or nutritional ones. Therefore, it requires a suitable and fine gene regulation control. In trypanosomatids, gene regulation occurs mainly at the post-transcriptional level. The expression of mRNAs is attained via regulation of their processing, transport, stability, and translation, mediated by the recognition of *cis* elements by RNA-binding proteins (RBPs). Alba-domain proteins are a conserved DNA/RNA-binding proteins family found in Archaea and Eukarya domains. This superfamily of proteins has been split into three major branches that include Archaea proteins and two eukaryotic-specific branches: the Rpp25/Mdp2 group and the Rpp20/Pop7 group. In *Leishmania infantum* and *Trypanosoma brucei* the Alba-proteins of Rpp25/Mdp2 group are involved in translation repression and in the regulation of gene expression during protozoan development. In *T. cruzi*, there is evidence that TcAlba30 protein may aggregate into cytoplasmic foci in parasites submitted to nutritional stress. This protein interacts with β-amastin390 mRNA and its overexpression resulted in the decrease of amastin mRNA levels, suggesting that this protein regulates the β-amastin390 expression. However, the role of other proteins of Rpp25/Mdp2 group and the proteins of Rpp20/Pop7 group identified in the genome of *T. cruzi* are still poorly understood. Therefore, the aim of the present work was to characterize the Alba protein family of *T. cruzi* and its RNA-binding capacity as an approach to uncover its functionality. *In silico* analyzes revealed that the genome of *T. cruzi* strains Brazil and Dm28c (TcI), Y (TcII), CL Brener Esmeraldo-like and TCC (TcVI) has four Alba genes, two for Rpp20/Pop7 group (TcAlba1 and TcAlba2) and two for Rpp25/Mdp2 group (TcAlba30 and TcAlba40). These genes code for highly conserved proteins in *T. cruzi*, but also among other Trypanosomatidae family members. The four proteins have physicochemical and structural characteristics compatible with RBPs. Particularly, the TcAlba30 and TcAlba40 proteins bind *in silico* to two RNA motifs: the Musashi binding element (MBE) and the Sxl binding site (SBS). *In vitro* interaction RNA-Protein assays using a representative of each Alba-family group (TcAlba2 and TcAlba40) showed that TcAlba40 protein, but not the TcAlba2, directly interacted with the 5' UTR region of the *kLYT1* transcript, where a MBE element is found. Likewise, transcription and translation expression of each protein were analyzed in the three *T. cruzi* stages, and the results showed that TcAlba2 and TcAlba40 mRNA are expressed in all stages. Nevertheless, expression of both genes is higher in the trypomastigote form compared to the epimastigote and amastigote forms, being approximately 2-fold for TcAlba2 and 3-fold for TcAlba40. Also, TcAlba proteins family were detected in all three stages, suggesting a continuous expression throughout *T. cruzi*'s life cycle. TcAlba1/2 were constitutively expressed, whereas TcAlba30/40 were mainly expressed in the parasite's replicative stages. Our results suggesting that TcAlba30/40 proteins from Rpp25/Mdp2 group are RBPs and them function may be related to biological processes associated with stages of development of *T. cruzi*.

Keywords: Alba-domain proteins, RNA motifs, RNA-binding proteins, *T. cruzi*

Palabras clave: proteínas Alba, motivos ARN, proteínas de unión a ARN, *T. cruzi*

Transcriptional changes during *Trypanosoma cruzi* metacyclogenesis

Cambios transcripcionales durante la metaciclogénesis de *Trypanosoma cruzi*

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During its life cycle, *Trypanosoma cruzi* undergoes different modifications and one of them is the change from epimastigotes to metacyclic trypomastigotes, known as metacyclogenesis. This differentiation stage is

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essential because the parasite acquires the form to infect humans and develop the disease. In this work, the transcriptome of metacyclic trypomastigotes and epimastigotes was analyzed to identify differentially expressed genes that may be involved in metacyclogenesis. Briefly, *in vitro* induction of metacyclogenesis was performed to obtain metacyclic trypomastigotes from a culture of epimastigotes. RNA-seq in triplicate from metacyclic trypomastigotes and epimastigotes was performed using Illumina/NovaSeq PE150. We implemented a genome reference-based approach using the Dm28c strain to assemble the transcriptome and differential gene expression analysis was done using DESEq2. Gene ontology analysis was performed using Tritrypdb. According to the RNA-seq results, we identified 17,120 total genes. 513 genes were differentially expressed in metacyclic trypomastigotes, 221 were up-regulated and 292 down-regulated. The analysis showed that these genes are related to relevant biological processes in metacyclogenesis. Within these processes, we found that most of the genes associated with infectivity and gene expression regulation were up-regulated in metacyclic trypomastigotes. Instead, the genes involved in cell division, DNA replication, differentiation, cytoskeleton, and metabolism, were mainly down-regulated. The results obtained in this work generate new knowledge about the biology of *T. cruzi*, applied to the understanding of differentiation and parasite-host interaction. In the long term, these genes could be used as potential therapeutic targets in the design of new drugs for Chagas disease.

Keywords: *T. cruzi*, metacyclogenesis, differentiation, transcriptome, RNA-Seq

Palabras clave: *T. cruzi*, metaciclogénesis, diferenciación, transcriptoma, ARN-Seq

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Replication origins in *Trypanosoma cruzi*: genome location and possible epigenetic regulation

Orígenes de replicación en *Trypanosoma cruzi*: ubicación en el genoma y posible regulación epigenética

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DNA replication must be closely controlled to ensure reliable maintenance of the genome in daughter cells. On the other hand, it is a process that can result in mutations. The balance between a careful process and an error-prone one results in higher or lower genetic variability. In *Trypanosoma cruzi*, the etiological agent of Chagas disease, DNA replication is tightly regulated since it is activated or deactivated during its life cycle. Moreover, DNA replication might contribute for the genetic variability, contributing for the success of the infection in the mammalian host. In a previously work, using MFA-seq methodology, we have been showed the presence of DNA replication origins within genes of the DGF-1 family, functional and pseudogenes predicted to encode surface proteins that might contribute to cellular invasion and/or escape from immunity. The presence of origins inside genes is a peculiarity of *T. cruzi* and made us hypothesize that it could contribute for replicative stress in these regions due the frontal collision between transcription and replication machineries (since firing of origins implies in forks emerging in both directions inside the genes and thus one of them will frontal collide with transcription). These replicative stresses might result in increased single-stranded DNA gaps and DNA double strand break (DSB) formation. Then, we also hypothesized that these replicative stresses could be the cause of homologous recombination favored in subtelomeric regions that are enriched by DGF-1 and also trans-sialidase genes. Here, using a single-molecule technique (nanopore-based sequencing followed by D-Nascent analysis) we were able to

detect replication origins fired in every single molecule and therefore we could detect facultative origins and compare them with constitutive origins (fired in many cells of a population) detected by MFA-seq. Using this approach, we could detect origins in other multigenic families besides DGF-1, strongly suggesting that in *T. cruzi* multigenic families contribute with DNA replication and that the presence of origins in these regions might indeed contribute for the genetic variability of these families. In addition, we co-related the presence of origins with the chromatin organization and our data point to a possible epigenetic control of replication firing, evidencing an involvement of chromatin organization with DNA replication in this parasite.

Keywords: Replication, replication origins, *Trypanosoma cruzi*, epigenetic

Palabras clave: Replicación, orígenes de replicación, *Trypanosoma cruzi*, epigenética

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Dynamics of the *Trypanosoma cruzi* genome

Dinámica del genoma de *Trypanosoma cruzi*

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The *Trypanosoma cruzi* parasite is able to invade almost any cell type, circulate freely in the blood or extracellular matrix, pass through the digestive tract of its insect vector and survive after being eliminated in the feces. It is also capable of infecting many mammals, including humans. This versatility of invasion and infection gives this parasite a virtually unique characteristic when compared to other pathogens. To cope with these characteristics requires a great adaptive capacity, and a fine regulation of gene expression, to allow adaptation to such different environments. This seems to go in the opposite direction of what is usually known about these parasitic protozoa, that they do not present the classical eukaryotic gene regulation mechanisms, and that their tendency is to transcribe most of the open reading frames, proceeding later to a post-transcriptional regulation. The surface envelope of *T. cruzi*, which is in a certain way its "letter of introduction" to the different cell types and/or immune systems they face, may constitute one of the explanations for the broad spectrum of infective capacity of these parasites. This surface is mainly constituted by three protein families encoded by hundreds of genes: mucin-like proteins (MUC), transialidases (TS) and mucin-associated proteins (MASP). They are GPI-anchored, with MUC and TS having been extensively studied, while MASP were discovered more recently, when the genome sequence of *T. cruzi* was first determined. An additional feature is that their genes are in the same regions of the genome. Thus, the *T. cruzi* genome consists of a core compartment (CC) with a high degree of synteny with the rest of the trypanosomatids (*Leishmania* spp., African trypanosomes), and a disruptive compartment (DC) where synteny with these species is lost. There are disruptive chromosomes (DC>90%), core chromosomes (CC>90%) and mixed chromosomes. Analyzing the steady state levels of mRNA we see that CC presents high and medium levels of expression -independently of the stage- and DC presents low levels of expression, also in all stages. On the other hand, the analysis of differentially expressed genes shows that in the CC they are distributed similarly in the different stages, while the CR shows highly significant differential expression in the trypomastigote stage, indicating that it is a genome specialized in the host-pathogen interaction. These different expression characteristics between compartments are due to epigenetic mechanisms and chromatin organization: chromosomal interactions, chromatin condensation,

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nucleosome positioning and methylation patterns, which seem to be determinant in the properties of each compartment.

Keywords: *Trypanosoma cruzi*, genomics, transcriptomcs, epigenetics

Palabras clave: *Trypanosoma cruzi*, genoma, transcriptoma, epigenética

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Nuclearly encoded mitochondrial genes and their role in the adaptation to mechanical transmission in African trypanosomes

Rol de los genes mitocondriales codificados en el núcleo en la adaptación de los tripanosomas africanos a la vida anaeróbica

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African trypanosomiasis comprises a group of diseases caused by various species of Trypanosoma of the Salivaria group, which affect both humans (sleeping sickness) and domestic and wild animals (Nagana). *Trypanosoma vivax*, the main cause of Nagana, is naturally vectored by the tsetse fly, whose distribution is restricted to the sub-Saharan zone of the African continent. In the proboscis of these insects, *T. vivax* undergoes a stage of its life cycle (epimastigote) of rapid cell division. During this replicative phase, trypanosomes need to carry out oxidative phosphorylation, due to the lack of nutrients in this environment. In contrast, in the stage of the life cycle that occurs in mammalian blood (rich in glucose), glycolysis is the almost exclusive source of energy supply. Thus, the respiratory chain plays a key role exclusively during passage through the insect vector. *T. vixax* was introduced to the American continent several centuries ago, where due to the absence of its natural vector (the tsetse fly), these parasites are transmitted by several species of hematophagous flies (Tabanidae and Stomoxys). These flies function as exclusively mechanical vectors (similar to an "infected needle"), so the parasite does not go through the replicative epimastigote phase that depends on mitochondrial respiration. This absence of respiratory requirement led us to propose that the mitochondrial genome of *T. vivax*, which encodes some protein subunits of the respiratory chain, should have undergone changes in response to the new lifestyle based on mechanical transmission. Analysis of the mitochondrial genomes of Venezuelan and one African strain (Y486) allowed us to identify several loss-of-function mutations (reading frame shifts). The mitochondrial editing system of these trypanosomes was drastically reduced in American *T. vivax*, with loss of guide RNAs linked to most of the mitochondrial genes that require such modifications. Expanding the range of American strains, we observed that most likely the incursion into America occurred several times, and in each of these independently occurred degradation of the coding capacity of the mitochondrial genomes. We then analyzed the adaptation to mechanical transmission of mitochondrial genes encoded in the nucleus (about 700 genes) and it is to be expected that many of these genes are changing in response to this drastic "environmental" modification. This same process analyzed in several strains of *T. vivax* that have acquired adaptation to mechanical transmission independently, as well as in *T. evansi*, offers the opportunity to study an adaptive process occurring simultaneously and independently in several lineages. Another affected system is the variable surface protein (VSG) coding system, associated with antigenic variation, since mechanical transmission implies not going through the phase in which parasites perform recombination (epimastigote). This surely affects the capacity to generate variability and has promoted important changes, which is reflected

in the fact that some American strains present a substantial reduction of genomic regions encoding VSG genes.

Keywords: *Trypanosoma vivax*, mechanical transmission, editing

Palabras clave: *Trypanosoma vivax*, transmisión mecánica, editing

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Deciphering the structure of *Trypanosoma cruzi* nuclear multiprotein complexes

Descifrando la estructura de complejos multiproteicos nucleares de *Trypanosoma cruzi*

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Chromatin structure is maintained by a series of multiprotein complexes that regulate DNA transcription, replication and repair. Recently, different components of these complexes have been identified by immunoprecipitation and proteomic analyses. However, given the low sequence identity it is not straightforward to identify some components by orthology methods or to predict the interactions between them. By an artificial intelligence approach using Rosetta-Fold and Alpha-Fold2 we were able to predict the structure of two subcomplexes containing the bromodomain proteins BDF5 and BDF6. The predicted interactions were corroborated by double hybrid assays in yeast and a working pipeline was established that will allow the prediction of larger complexes. BDF5 is essential and would function as a general regulator of transcription, in a complex with three bromodomains and Histone Acetyl Transferase activity. On the other hand, BDF6 is part of a TinTin-like complex, a subcomponent of the NuA4 complex, and is essential for trypomastigote infectivity and the development of intracellular amastigotes.

Keywords: *Trypanosoma cruzi*, multiprotein complexes, BDF5, BDF6

Palabras clave: *Trypanosoma cruzi*, complejos multiproteicos, BDF5, BDF6

Origin and evolution of Eukaryotes Telomeres: the organization *Leishmania* and *Trypanosoma cruzi* telomeres

Origen y Evolución de los Telómeros: organización de los telómeros en *Leishmania* y *Trypanosoma cruzi*

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Very early in the evolution of eukaryotic organisms, linear chromosomes appeared and with them arose the need to protect their ends to avoid being treated as breaks or accidents, and to solve the problem of the culmination of the replication of the delayed chain (lagging strand). The appearance of the telomeric structure (telomeres and associated proteins) served to attack the first problem, while the second was solved with the telomerase enzyme acting in concert with the proteins associated with telomeres. The region between the first autosomal gene and the telomere is called subtelomere, which is characterized by being genetically unstable, allowing homologous and heterologous interchromosomal recombination without compromising the synteny of the rest of the chromosome. In some organisms such as *Trypanosoma brucei, Plasmodium falciparum*, and *Saccaromyces cereviseae* these regions play a crucial role in surface antigenic variation or selective gene silencing. In our studies we have found a very particular telomeric structure in *Leishmania major* which evokes a possible telomerase-independent mechanism, and a complex subtelomere formed by sequence blocks. Whereas in *Trypanosoma cruzi* there may be coding genes (and their pseudogenes) very close to the telomere mostly belonging to multigene families such as RHS, DGF.1, Transialidases and abundant non-LTR retrotransposons. Our experiments have demonstrated the ability of these subtelomeres to make non-homologous interchromosomal recombination and this fact added to the great diversity present in these subtelomeres has led us to postulate that these subtelomeres are sites for the generation of genetic variability to confer an adaptive advantage to these parasites. In this lecture we will review the telomeric structure and a comparative analysis between the telomeres of Trypanosomatids and the rest of eukaryotic organisms.

Keywords: telomeres, evolution, parasites, genome dynamics, *Trypanosoma cruzi, Leishmania*

Palabras clave: telómeros, evolución, parásitos, dinámica genómica *Trypanosoma cruzi, Leishmania*

Signaling and adaptive mechanisms in trypanosomatids: a possible Achilles heel in the fight against trypanosomiasis

Señalización y mecanismos adaptativos en tripanosomátidos: un posible talón de Aquiles en la lucha contra la tripanosomiasis

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Trypanosoma cruzi is the etiological agent of Chagas disease or American trypanosomiasis, a neglected tropical condition historically endemic to Latin America that, due to human migration, has spread beyond its traditional boundaries. It is now estimated that 6 to 8 million people suffer from this disease worldwide. *T. cruzi* is a protozoan parasite that presents a complex life cycle, during which it must cope with sudden changes in the environment to which it must respond immediately to survive. Cyclic nucleotide phosphodiesterases have been implicated in the proliferation, differentiation and osmotic regulation of trypanosomatids. In some trypanosomatid species they have been validated as molecular targets for the development of new therapeutic agents. It has also been shown that many inhibitors of mammalian PDEs lack an inhibitory effect on trypanosomatid enzymes, indicating that there are differences in the substrate specificity of parasite and host enzymes, foreshadowing the possibility of identifying compounds that selectively inhibit the parasite enzyme. The limited possibilities to edit *T. cruzi* genome have made it difficult to carry out knockout and knockdown

experiments, and drug target validation has been limited to biochemical evaluations. Recently, the development of CRISPR/Cas9 as a tool for genomic edition brought a new perspective to the study of *Trypanosoma cruzi*. According to the most often applied protocols, epimastigotes are co-transfected with a single plasmid bearing both the gene for Cas9-GFP expression and a sequence to be translated into a single guide RNA (sgRNA), jointly with a lineal donor DNA encompassing a selection marker flanked by sequences homologous to the target gene. Here, we tested an alternative approach for the generation of Phosphodiesterase (PDE) knockout parasites. We obtained epimastigotes from Tul II strain stably expressing Cas9-GFP in the nucleus in all parasite stages, with no detrimental effects on epimastigote growth or differentiation nor on trypomastigote infection capability. These Cas9-GFP epimastigotes were co-transfected with the sgRNA + DNA donor pair, according to the intended gene target. sgRNA were obtained by *in vitro* transcription using a template DNA bearing the specific + scaffold sequence under a T7 promoter. To obtain the donor DNA we designed a pre-donor formed by a sequence including several restriction enzyme recognition sites flanked by 30-bp arms homologous to the sequence adjacent sgRNA annealing target. This pre-donor allowed to easily generate a variety of donor DNAs by cloning alternative selection markers. DNA extracts (boiling-preps) from 4-day post-transfection cultures were evaluated by PCR using mixed primer pairs: while one of the primers annealed to the target gene, the second primer annealed to a sequence in the donor DNA, allowing assessment of its correct insertion in the gene of interest. Advantages of this take on CRISPR/Cas9 edition include its versatility for choosing and switching between alternative selection markers and a quick and affordable generation of the components of the system and analysis of the transfected cultures, while possibly facilitating complementation assays on the KO lines. Finally, our results indicate that loss of PDEs (even partial) is incompatible with normal parasite function/parasite viability.

Keywords: *Trypanosoma cruzi*, Chagas disease, signal transduction, phosphodiesterases, CRISPR, drug discovery

Palabras clave: *Trypanosoma cruzi*, enfermedad de Chagas, transducción de señales, fosfodiesterasas, CRISPR, descubrimiento de drogas