RT.01 – GENOMICS OF TRYPANOSOMATIDS

RT.01.1 - **UNTANGLING COMPLEXITY IN TRYPANOSOME GENOMES**

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Although the genomes of trypanosomatids are relatively small (haploid size less than 60 MB) their complete and accurate assemblies, as well their annotation have proven to be not trivial. These difficulties are specially pronounced in the case of Trypanosoma cruzi, whose genome sequence remains in a draft (and fragmented) status in spite that it was first made available more than 10 years ago. The intrinsic complexity of this parasite's genome relies on the abundance of repetitive sequences and genes organized in tandem, which has hindered high-quality genome assembly and annotation. This poses several limitations for diverse types of analyses that require high degree of precision. Several technologies are now available to tackle these issues. Long reads generated by third-generation sequencing technologies (PACBIO and MinION) are particularly suitable to address the complications associated with T. cruzi's genome since they permit direct determination of the full sequence of large clusters of repetitive sequences without collapsing them. This, in turn, not only allows accurate estimation of gene copy numbers but also circumvents, to a large extend, assembly fragmentation. Chromosome level scaffolding, however, remains incomplete even with these approaches. Alternative technologies, including optical and interaction maps (Hi-C), can fill this gap. The analysis of the genome sequences of two T. cruzi clones: the hybrid TCC (TcVI) and the non-hybrid Dm28c (TcI), determined by using these technical advances allowed to produce significant improvements in the assemblies since they enabled us to accurately estimate gene copy numbers, abundance and distribution of repetitive sequences (including satellites and retroelements). Novel tandem and dispersed repetitive sequences were identified. In the case of hybrid clones, homologous chromosomes were separately assembled (parental haplotypes were retrieve as separate contigs instead of a unique mosaic sequence). Besides, manual annotation of surface multigene families, mucins and trans-sialidases seems to be mandatory to get a better view of these complex groups of genes.

RT.01.2 - **COMPARATIVE GENOMICS REVEALS RECOMBINATION EVENTS LEADING TO ANTIGENIC VARIATION IN *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi is the causative agent of American trypanosomiasis (Chagas disease). The genome of the parasite is highly repetitive. It contains several thousand of closely related copies of genes encoding a highly diverse repertoire of surface molecules, with roles in cell invasion, immune evasion and pathogenesis. Complete genome sequencing was therefore previously impossible and the available reference sequences were incomplete and fragmented. By producing complete genome assemblies for a *T. cruzi* one (TcI) and a TcII strain using PacBio high coverage single molecule sequencing, we we have been able to study surface molecule genes and their importance for immune evasion and pathogenesis in detail. The organization of the surface molecule genes and the overrepresentation of repeat elements in subtelomeres suggest mechanisms for the generation of novel surface molecule variants. In a large comparative genomics effort, including comparative analysis of 35 *T. cruzi* TcI isolates and clones from different geographic locations, sample sources and clinical outcomes and TcI field isolates from Ecuador, as well as additional strains from other *T. cruzi* clades, we have identified recombination events that produce novel surface molecule variants. This will make it possible to systematically study mechanisms whereby this parasite evades the immune system and causes chronic infections. **Supported by: Keywords:** Trypanosoma cruzi; comparative genomics; surface molecule variants

RT.01.3 - **GENETIC DIVERSITY AND COMPARATIVE GENOMICS OF TRYPANOSOMA CRUZI: PROGRESS, CHALLENGES, AND WIDER PRIORITIES.**

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High throughput and high read length (PacBio) genome sequencing has revolutionised the ability to resolve and compare parasite genomes. Nevertheless, for Trypanosoma cruzi high fidelity genome assembly remains challenging, especially due to the extremely repetitive nature of multiple gene families. Of the six or seven recognised genetic lineages of T. cruzi (TcI-VI; TcBat) imperfect reference assemblies are available for four, and there are few studies of multiple genomes from single or comparative foci of transmission. Thus far, this research has, for example, given insight into phylogenetic relationships, indicated a functional mechanism among complex gene families [1] and confirmed the TcII/TcIII hybrid nature of TcV and TcVI, which have spread rapidly throughout the southern cone region with the vector Triatoma infestans. In contrast to Leishmania and Trypanosoma brucei, only a single experimental genetic cross of T. cruzi has been achieved [2], yielding hybrids with approximately 4N DNA content, which suffered genome erosion towards 3N with prolonged maintenance in vitro [3]. Historically, T. cruzi was considered to be clonal but this was based on limited, disparate isolate sampling. It is apparent that T. cruzi retains an epidemiologically important capacity for genetic exchange but the meiotic and potentially non-meiotic (parasexual) mechanisms in natural populations are not elucidated.

Since 1981 [4] it was proposed that the differential outcome of human T. cruzi infection, particularly the skewed prevalence of megasyndromes in the southern cone compared to northern South America and Central America, was partially dependent on the differential distribution of TcI and TcIV (in northern South America and Central America) and TcII, TcV and TcVI (southern cone). However, this remains unproven. To facilitate identification of patients carrying TcII or TcV/TcVI, and to overcome difficulties in isolating representative genotypes from tissue-sequestered T. cruzi populations, we exploited genetic diversity within the TSSA gene to develop a lineage-specific point-of-care (POC) rapid diagnostic test (RDT). Recognition of TcII/V/VI specific synthetic peptide antigens was associated with higher risk of severe Chagas disease [5]. We proposed that this may be explicable by higher inflammatory response leading to more severe disease and boosting antibody to the TcII/V/VI epitope. The same RDT was designed to be directly applicable to the discovery of reservoir hosts and to define transmission cycles [6, and in preparation].

As yet, benznidazole or nifurtimox are the only treatment options for Chagas disease, although intensive drug discovery for better alternatives is in progress, largely under the auspices of DNDi, and with the aid of in vivo bioluminescent imaging to evaluate cure [7]. Although not straightforward, comparative genomics of resistant clones derived from clonal susceptible strains can potentially reveal resistance mechanisms and guide new chemotherapeutic strategies.

Despite the immense fascination and potential of genomics and bioinformatics analysis, a wider priority for Latin America has to be dealing with the persistent vector borne transmission of T. cruzi in the Gran Chaco region, and resurgent Chagas disease where control programmes falter.

1. BioRxiv 283531; doi: https://doi.org/10.1101/283531; 2. Nature. 2003; 421(6926): 936-9; 3. Int J Parasitol. 2009; 39(12):1305-17; 4. Lancet. 1981; 1(8234):1338-40; 5. Clin Infect Dis. 2018; 67(4):519-524; 6. Parasit Vectors. 2016; 9(1):584-63; 7. Antimicrob Agents Chemother. 2015; 59(8):4653-61.

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Keywords: Trypanosoma cruzi; genetic exchange; lineage-specific rapid diagnostic tests

RT.01.4 - **Exploring the content of ncRNAs in the protozoan parasite *Leishmania***

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Several classes of ncRNAs have been revealed in recent years. ncRNAs are involved in a variety of regulatory processes in a wide range of organisms. Our laboratory if focused in understanding some of the layers at which regulation of gene expression occurs in *Leishmania*. Serendipitously, studying a group of short unannotated and polyadenylated transcripts from *Leishmania major*, we identified and partially characterized one of them, *ODD3*, arising from the 3'UTR of one of the two copies of a ribosomal protein gene (RPS16). *ODD3* occurs as an independent ~150 nucleotides-long transcript. We extended the search for similar UTR-born or uaRNAs to *Leishmania donovani* exploring non-polysomal ribonucleoprotein and we further investigated the presence and diversity of ncRNAs in *Leishmania*, after deep RNA sequencing total RNA from procyclic and metacyclic promastigotes and axenic amastigotes, the main lifecycle stages. A pipeline for detection of transcripts with a possible ncRNA function was developed and a combination of 5 different ncRNA predictors were used to identify 11,376 putative ncRNAs, distributed in different categories. Moreover, comparative analysis of each transcriptome allowed detection of differentially expressed genes (DE genes). Among the differentially expressed ncRNAs, with a fold change >1.5, a total of 295 transcripts were DE in all the 3 pairwise comparisons. Northern blotting analysis confirmed the presence of transcripts of similar length. Our results suggest that ncRNA and possibly UTR-transcripts exist in *Leishmania* and their roles will be investigated. Knockout and tagging of some of these transcripts were obtained using CRISPR/Cas9 editing machinery and phenotypes are under analysis.

RT.02 – AMOEBA, GIARDIA, AND TRICHOMONADS: EVOLUTION AND PATHOGENESIS

RT.02.1 - **PHYLOGENOMICS, ANCESTRAL STATE RECONSTRUCTION AND GENOME EVOLUTION IN SHELLED AMOEBOZOA.**

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The Arcellinida are an ecologically important and biodiverse group of shelled amoeboid microbes. This microbial taxon has a rich fossil record, dating to 750 MYA (Neoproterozoic), making them are a key lineage to link extant organismal diversity with earth history. However, their phylogenetic history is still unresolved. I will present a phylogenomic reconstruction of the Arcellinida based on 20 taxa. Upon the phylogenetic framework, we have performed ancestral state morphological reconstructions to reveal putative affiliations of fossils. We demonstrate that the crown group of Arcellinida was already diversified before the Neoproterozoic. The most likely event involved with this early diversification was the deeper ocean oxygenation in the Neoproterozoic. In contrast to current hypothesis, this indicates that the diversification of the stem lineages of eukaryotes must have happened earlier in the evolutionary history of eukaryotic life, driven by a yet undetermined biogeochemical event. In addition, the sequencing of this rich taxonomic assemblage has given insights into the molecular evolution of the group, I will present some of the questions that are currently being work on in my laboratory. **Supported by:**FAPESP **Keywords:** Phylogenomics; ancestral state reconstruction; evolution

RT.02.2 - **PATHOPHYSIOLOGY OF GIARDIASIS: FROM PROTEOMICS TO PATHOGENESIS**

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Giardia is a protozoan parasite of public health relevance that causes gastroenteritis in a wide range of hosts. Two genetically distinct lineages (assemblages A and B) are responsible for the human disease. Although it is clear that differences in virulence occur, the pathogenesis and virulence of Giardia remain poorly understood. The genome of Giardia is believed to contain open reading frames that could encode as many as 6000 proteins. By successfully applying quantitative proteomic analyses to the whole parasite and to the supernatants derived from parasite culture of assemblages A and B, we confirm expression of~1600 proteins from each assemblage, the vast majority of which are common to both lineages. To look for signature enrichment of secreted proteins, we considered the ratio of proteins in the supernatant compared with the pellet, which defined a small gro! up of enriched proteins, putatively secreted at a steady state by cultured growing trophozoites of both assemblages. This secretome is enriched with proteins annotated to have N-terminal signal peptide. The most abundant secreted proteins include known virulence factors such as cathepsin B cysteine proteases and members of a Giardia superfamily of cysteine-rich proteins that comprise variant surface proteins, high-cysteine membrane proteins, and a new class of virulence factors, the Giardia tenascins. We demonstrate that physiological function of human enteric epithelial cells is disrupted by such soluble factors even in the absence of the trophozoites. We are able to propose a straightforward model of Giardia pathogenesis incorporating key roles for the major Giardia-derived soluble mediators. **Supported by:**European Commission Framework VII **Keywords:** Giardiasis; pathogenesis; virulence factors

RT.02.3 - **STUDIES ON THE GIARDIA INTESTINALIS CELL CYCLE**

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The lifecycle of Giardia alternates between the motile trophozoite and the infectious cyst. The proliferation of Giardia trophozoites during an active infection, and the restriction point for the differentiation of trophozoite to cyst are dependent on the tight regulation of the cell cycle. Despite the importance of the cell cycle in Giardia pathogenesis, it has been difficult to study this process due to the absence of a synchronization procedure that would not induce cellular damage resulting in artefacts. We utilized counterflow centrifugal elutriation (CCE), a size based separation technique, to successfully obtain fractions of Giardia cultures enriched in defined cell cycle stages. These fractions were subjected to RNA-seq to obtain a transcriptome of the Giardia cell cycle. In addition, samples from Giardia cultures synchronized by aphidicolin were used in microarray analysis. Comparison of the RNA-seq and the microarray results identified a core set of genes that are differentially expressed in G1/S and G2/M. These genes include the cyclins, CDKs, thymidine kinase, MCM5, polo-like kinase, and Proliferating Cell Nuclear Antigen. We also observed an up-regulation of genes encoding proteins that are associated with the ventral disc (i.e. beta-giardin, SALP-1 and delta-giardin) in G1/S, which suggest that the synthesis of this important and complex structure initiates early in the cell cycle. However the RNA levels of these genes during the cell cycle vary less than 4 to 5 fold, which might indicate that large changes in gene expression are not required by Giardia to regulate the cell cycle. We also observed an increase in the appearance and size of the median body in the cells from elutriation fractions corresponding to the progression of the cell cycle from G1 to G2/M. Consequently, CCE could be used to examine the dynamics of the median body and other structures and organelles in the Giardia cell cycle. **Supported by:**National Science and Engineering Research Council (NSERC) of Canada **Keywords:** Rna-seq; microarray; median body

RT.02.4 - **PURINERGIC SIGNALING IN TRICHOMONAS VAGINALIS-HOST INTERACTION**

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Trichomonas vaginalis, a parasitic protozoan, is the etiologic agent of trichomoniasis, the most prevalent non-viral sexually transmitted disease worldwide. The spectrum of clinical trichomoniasis in women ranges from the asymptomatic state to flagrant vaginitis and may cause major health consequences, including atypical pelvic in inflammatory disease, cervical and prostate cancers, low-weight and premature birth. Importantly, the disease is also a co-factor in promoting transmission of HIV. Metronidazole and tinidazole, both 5-nitroimidazoles, are two drugs of choice recommended by Food and Drug Administration (FDA, USA) for the treatment of trichomoniasis. However, drug-resistant isolates of T. vaginalis have been reported and therapeutic alternatives are being researched. The investigation of biochemical aspects of the parasite and its relationship with the host can help to clarify some mechanisms of trichomoniasis pathogenesis. Adenosine 5'-triphosphate (ATP) plays a crucial role in many extracellular functions and can act as damage-associated molecular pattern (DAMPs) performing a proinflammatory function in the microenvironment of damaged cells. In the other hand, adenosine may revert some of effects induced by extracellular ATP through immunosuppressive modulation. Both ATP and adenosine play their effects by binding to specific receptors named purinoceptors, P2 and P1, respectively. The regulation of this cell signaling can be attributed to enzymes called ectonucleotidases: the ectonucleoside triphosphate diphosphohydrolase (E-NTPDase) family (NTPDase 1-8) and ecto-5′-nucleotidase (CD73). In sequence to ecto-5'-nucleotidase activity, adenosine deaminase (ADA) catalyzes the conversion of adenosine to inosine. In parasites the purinergic system represents an important mechanism of escape of host immune response by ATP degradation and adenosine production, and in this manner modulating immune response. Moreover, in spite of adenosine importance in limiting the inflammatory response, pathogens may scavenge adenosine for growth from host cell because these organisms lack the ability to synthesize the purine ring de novo. NTPDase, ecto-5'-nucleotidase and ADA activities have already been characterized in T. vaginalis trophozoites. Our group have shown the importance of these enzymes for the parasite, since in an environment with low concentrations of adenosine the enzymes NTPDase and ecto-5'-nucleotidase provide the nucleoside necessary for the parasite growth. In addition, adenine nucleotides (ATP, ADP and ATPγS) as well as ATP enzymatic hydrolysis were not decisive for NO release by T. vaginalis-stimulated neutrophils. Unlike ATP, adenosine and inosine decreased significantly the NO levels, revealing the immunosuppressive effect of adenosine – promoted by A2A activation - and the importance of ecto-5'-nucleotidase activity of T. vaginalis during the establishment of trichomoniasis. Moreover, when comparing the effects of nucleosides, adenosine, but not guanosine, protects vaginal epithelial cells from T. vaginalis cytotoxicity. Considering the high concentration of extracellular ATP at the infection site, the purinergic system represents a primordial form of chemical intercellular signaling where the parasite's ectonucleotidases play an important role. The enzymes hydrolyze ATP producing adenosine which will be uptake and employed for the parasite growth and moreover, the anti-inflammatory effects of the nucleoside can contribute to the effectiveness of infection. In this context, the enzymes may be considered interesting targets of new alternatives for the treatment of trichomoniasis.
Supported by:CNPq, FAPERGS **Keywords:** Trichomonas vaginalis; adenosine; pathogenicity

RT.03 – RECENT INSIGHTS ON INVASION, VIRULENCE, CELL BIOLOGY AND PATHOGENESIS OF APICOMPLEXANS

RT.03.1 - **AN UPDATED LOOK ON TOXOPLASMA GONDII BEHAVIOR ALONG ITS INTRACELLULAR CYCLE**

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Investigations in Toxoplasma gondii and toxoplasmosis have been the focus of research of several groups since its first description, in 1908. The ultrastructure of its forms and intracellular development has been extensively described in the years 60's and 70's. The initial ultrastructural model has been improved since then in an effort to bring together the organelle dynamics, the molecules that have been identified and the behavior of the parasite. In this sense, combining new approaches of sample preparation, molecular tools, innovative technology of observation and data processing we propose that the tachyzoite of Toxoplasma gondii undergoes a sequence of ultrastructural modifications during the invasion, the establishment and development in the parasitophorous vacuole and, at last, in the egress or, alternatively, in the conversion to bradyzoites. Many aspects of this journey are still misunderstood, raising exciting discussion and opening gaps to be hopefully filled in the years to come. **Supported by: Keywords:** Toxoplasma gondii; ultrastructure; cell biology

RT.03.2 - **INSIGHTS INTO THE RHOPTRY SECRETION MECHANISM DURING APICOMPLEXA INVASION**

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Together with ciliates and dinoflagellates, apicomplexan parasites belong to the Alveolata superphylum. Apicomplexan parasites are important pathogens of humans and domestic animals. They inclde Plasmodium spp., the causative agent of malaria, Toxoplasma, a prominent cause of human congenital infections, and Cryptosporidium, a leading cause of severe diarrhoea and mortality in infants. These parasites are motile and actively invade the cells in which they replicate. The ability of these parasites to cause disease depends on the coordinated secretion of specialized secretory organelles. The rhoptries are particularly important, because they act as the apicomplexan equivalent of bacterial secretion systems. They inject parasite proteins directly in the cytoplasm of host cells not only for invasion but also to hijack host functions crucial to establish and maintain infection. However, in contrast to bacteria where the secretion machinery has been resolved to atomic detail, how eukaryotic parasites secrete and inject rhoptry effectors into cells is an enigma. I will present insights into the docking and fusion machinery of rhoptry with the parasite plasma membrane.

Our study supports the hypothesis of an Alveolate conserved mechanism for rhoptry-dependent membrane fusion event. Understanding this mechanism will guide future efforts to disrupt parasite infection. **Supported by: Keywords:** Apicomplexa; rhoptry; secretion

RT.03.3 - **THE PARASITE KINASE ROP16 IS A KEY VIRULENCE FACTOR THAT ACTS IN CIS AND TRANS AND MEDIATES SUPPRESSION OF T CELL RESPONSES**

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The ability of Toxoplasma gondii to inject the kinase ROP16 into host cells results in the activation of the transcription factor STAT6, but it is unclear how these events impact this infection. Here, parasites that inject Cre-recombinase with their rhoptry proteins were used to distinguish infected macrophages from those only injected with parasite proteins. Transcriptional profiling revealed that injection of rhoptry proteins alone was sufficient to induce a STAT6-dependent M2 phenotype common to injected or infected cells, but only in infected cells was reduced expression of anti-microbial genes and antigen presentation pathways apparent. Further, deletion of ROP16 did not compromise the ability of T. gondii to replicate in macrophages in vitro or in immune-deficient mice, but in immune competent mice the loss of ROP16 resulted in reduced parasite numbers and heightened parasite-specific T cell responses. Thus, ROP16 is a virulence factor that can act in cis and trans to promote an M2 program and limit the magnitude of parasite-specific T cell responses.

RT.03.4 - **PATHOGENESIS OF SEVERE MALARIA**

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Proliferation and differentiation inside erythrocytes are important steps in the life cycle of Plasmodium spp. To achieve these, the parasites export polypeptides to the surface of infected erythrocytes; for example, to import nutrients and to bind to other erythrocytes and the host microvasculature. Binding is mediated by the adhesive polypeptides Plasmodium falciparum-encoded repetitive interspersed families of polypeptides (RIFINs), subtelomeric variant open reading frame (STEVOR) and P. falciparum erythrocyte membrane protein 1 (PfEMP1), which are encoded by multigene families to ensure antigenic variation and evasion of host immunity. These variant surface antigens are suggested to mediate the sequestration of infected erythrocytes in the microvasculature and block the blood flow when binding is excessive. Currently, there is no adjunctive drug for the treat¬ment of severe malaria, but this may change owing to our improving molecular understanding of the pathogenesis. Blocking merozoite invasion has not previously been proposed as an adjunctive therapeutic approach, but it may preclude the early expansion of an infection before exacerbated sequestration and death occurs. Interestingly, a negatively charged carbohydrate-based drug named sevuparin, which was developed from heparin, blocks merozoite invasion in vitro and desequesters pRBCs in different animal models. Sevuparin also showed promise in a clinical phase I/II study, in which it robustly blocked merozoite invasion and transiently desequestered pRBCs in patients with uncomplicated P. falciparum malaria. Beneficial effects of sevuparin were detected after only 1 h from when the study commenced and lasted for the first 6 h.

RT.04 – HOST PARASITE INTERACTION

RT.04.1 - **ARGININE MONOMETHYLATION EPIGENETICALLY REGULATES PARASITE VIRULENCE VIA THE MRNA-BOUND PROTEOME OF *LEISHMANIA*.**

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Background:
Constitutive transcription in *Leishmania* parasites results in gene regulation being overwhelmingly reliant on post-transcriptional mechanisms, yet strikingly few *trans*-regulator proteins are known or characterized. Protein aRginine MethylTransferase enzymes (PRMTs) catalyse arginine methylation in various cellular processes. *Lmj*PRMT7 is an R-monomethylating enzyme implicated in pathogenesis with unknown substrates.

Objectives:
Our primary objectives were to independently and quantitatively isolate, identify and analyze both the mRNA-binding proteome and whole cell proteome of the 3 main lifecycle stages in *Leishmania* as well as the protein targets of PRMT7 R-methylation.

Results:
Utilizing optimised crosslinking, stringent polyA-RNA isolation conditions and in-depth, quantified mass spectrometry, we present a comprehensive analysis of over 1,400 mRNA-binding proteins (mRBPs) from the three main *Leishmania* lifecycle stages. We then analyzed potential correlation between published transcript abundance and corresponding protein expression as well as stage-specific variation in protein expression versus RNA binding potential. Using comparative methyl-SILAC proteomics for the first time in protozoa, we identified 40 proteins hypomethylated in PRMT7-null mutants including 17 target RNA-binding proteins (RBPs). We find PRMT7 can modify RBPs Alba3 and RBP16 as direct substrates *in vitro* and PRMT7 knockout reduces both RBP16 protein half-life and Alba3 mRNA-binding capacity *in vivo*. RNA immunoprecipitation (RIP) analyses demonstrate PRMT7-dependent methylation promotes Alba3 association with target transcripts and thereby controls stability of *delta-amastin* surface antigen.

Conclusions:
These are the first studies to comprehensively and quantitatively identify the mRBPome and R-monomethylome of *Leishmania* parasites. Our results confirm previous studies which indicate that in *Leishmania* parasites, RNA levels are not a strong predictor of whole cell protein expression or RNA binding potential.
The novel role for PRMT7-dependent R-methylation of RBPs suggests a post-translational regulatory pathway controlling both gene expression and virulence in *Leishmania*. This work introduces *Leishmania* PRMTs as epigenetic regulators of mRNA metabolism with novel mechanistic insight into the dynamic *Leishmania trans*-regulatory mRNA:Protein (mRNP) complexes that drive the parasite's lifecycle progression.

Supported by: Medical Research Council **Keywords:** Rna binding proteins; prmt methylation; leishmania

RT.04.2 - *T. CRUZI* INTRACELLULAR DEVELOPMENT: CELLULAR AND MOLECULAR ASPECTS

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, has a variable clinical course ranging from asymptomatic to more severe cardiac, digestive or cardio-digestive commitment. Previous studies from our group have demonstrated that parasite differential tissue distribution in mice can be reproduced in vitro, at least in primary cultures of cardiomyocytes. These data show that the parasite tissue selection is a result of the direct parasite-host cell interaction and may be independent of the immune system. We also showed that the differential distribution is not only due to parasite cell invasion ability, but also due to parasites' mutiplication efficiency inside host cells. Recently, we showed that murine and human cardiomyocytes (CM) respond differently to infection with two different clonal populations of *T. cruzi*, JG and Col1.7G2, producing different levels of reactive oxygen species (ROS). JG infection of both CM cultures induces higher amounts of ROS when compared to those infected with Col1.7G2 and inhibition of oxidative stress decreases JG, but not Col1.7G2, intracellular multiplication. Additionally, we showed that Col1.7G2 produces more antioxidant enzymes, suggesting it is less affected by an oxidative environment. These results together suggested that, as observed before for macrophages, ROS production signals to the parasite, contributing to increasing its intracellular multiplication rate. However, little is known about how ROS-induced signaling occurs, as well as whether this signaling is only efficient for a group of parasite populations and / or whether their levels are determinants for such signaling. In parallel, we have also shown that parasites multiplies better in fibroblasts that lack lysosomal membrane proteins, LAMP-1 and 2. Most recently, we have further shown that parasites released from these LAMP deficient cells behave differently from those released from wild type fibroblasts, most likely due to differences in parasite surface protein expression. These parasites present different invasion rates, which were not related to their adhesion capacity, but most likely to its ability in inducing host cell calcium signaling. LAMP-deficient cells retain cholesterol in the intracellular medium which could contribute to the differential intracellular development of the parasites in these cells. In parallel this accumulation of cholesterol can alter the pH of lysosomes, which could contribute to changes in the intracellular environment where parasite temporarily resides. Altogether, these data indicate that changes in intracellular environment may interefer with parasite metabolism and replicative ability, as well as modulate the expression of parasite surface proteins. The more detailed characterization of these cellular colonization processes, the effects of oxidative stress and of modified environments, such as that of LAMP-deficient cells, can certainly better elucidate parasite-host cell interaction processes, contributing to a better understanding of the evolution of infection and, possibly, for the development of a more effective treatment. **Supported by:**CNPq, FAPEMIG **Keywords:** T. cruzi; oxidative stress; lamp

RT.04.3 - TGF-β AS A MULTIFUNCTIONAL PROTEIN IN T. CRUZI HOST-CELL BIOLOGY AND IN THE DEVELOPMENT OF CHAGAS HEART DISEASE

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The regulation of the formation and degradation of the extracellular matrix is the basis of the fibrotic processes and TGF-β stands out among the regulatory cytokines of these processes. Studies demonstrate the involvement of this cytokine in acute and chronic chagasic cardiopathy, with exacerbation of TGF-β plasma levels and the activation of its cell signaling pathway as peculiar aspects of patients in the more advanced stages of the disease, associated with cardiac fibrosis. In in vitro approaches of T. cruzi infection using SB431542, it is observed decreased invasion of T. cruzi in cardiomyocytes and fewer intracellular amastigotes per infected cell. These data encouraged us to test the proof of concept in experimental models in vivo. The effects of SB431542 and GW788388 (improved compound with oral application) were tested in an experimental model for acute phase of Chagas disease, and we observed a reduction in parasitemia and mortality levels, with lower tissue

inflammation and parasite load, and an improvement in electrical conduction in the heart. The blockade of TGF-β as a treatment for cardiac fibrosis during the chronic phase of experimental Chagas' disease has been recently performed by our group. We demonstrated that the inhibition of the TGF-β signaling pathway was effective for the treatment of cardiac fibrosis during the chronic phase of experimental Chagas' disease, although it is more complex compared to fibrosis observed in the acute phase. Recently, our group verified the association between the presence of single base polymorphisms in the TGF-β gene and the susceptibility to Chagas disease in the Brazilian population. We observed that the T-allele at position -509 and the C allele at codon 10 could be considered a relative risk factor for the development of Chagas' disease. In addition to the important role of TGF-β in the development of Chagas' disease, the participation of this molecule in basic cellular processes in T. cruzi biology is now evident as a regulatory molecule involved on different stages of its life cycle. It is known that T. cruzi requires activated Smad-dependent signaling pathway in response to TGF-β to invade the host cells, and is able to trigger this pathway during the invasion events. In addition, T. cruzi directly activates latent TGF-β, mainly by the action of cruzipain, which could contribute to the events of cellular invasion besides the exacerbation of the fibrosis process in Chagas' disease. According to the World Health Organization, Chagas disease is endemic in Latin America, affecting about 8 million people and approximately 25 million individuals are at risk of T. cruzi infection. In Brazil, Chagas' disease affects about 4 million people (Ministry of Health, 2017) and is a serious public health problem. Even after a centenary since the discovery of Chagas' disease, the treatment is still based mainly on two chemotherapeutics, benzonidazole and nifurtimox. Both have trypanosomicidal action with low efficiency in the chronic phase of the disease, in which the frequency of circulating parasites is lower. Interference in the factors involved in the development of the disease may lead to the proposition of therapeutic strategies that associate trypanosomicidal, anti-inflammatory, immuno-regulatory and anti-fibrotic agents. We hope that soon we would be able to contribute with new compounds that interfere in the development and / or reversion of cardiac fibrosis of Chagas disease, favoring a better quality of life for the thousands of chronic patients still neglected today.
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