RT.01 – GENOME MAINTENANCE AND RNA STABILITY IN TRYPANOSOMATIDS

RT.01.1 - **CONTROL OF *TRYPANOSOMA BRUCEI* DIFFERENTIATION BY RNA BINDING PROTEINS**

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Trypanosoma brucei multiplies in the blood and tissue fluids of mammals (as bloodstream forms) and the digestive system of Tsetse flies (as procyclic forms and epimastigotes). Since RNA polymerase II transcription is polycistronic, most regulation of gene expression affects *trans* splicing, translation, and mRNA decay. The fate of each mRNA is determined by the various proteins that are bound to it, and 3'-UTR sequences often have decisive roles. mRNAs encoding regulators, such as protein kinases and RNA-binding proteins, have abnormally long 3'-UTRs. For example, RBP10 is an RNA-binding protein that is expressed only in the bloodstream form, and which is required for maintenance of the bloodstream form differentiation state (PMID 28800584). The *RBP10* 3'-UTR is 7kb long and includes at least 5 separate regulatory regions specifying high expression in bloodstream forms or low expression in procyclic forms. RBP10 protein binds to $UA(U)_6$ in mRNA 3'-UTRs. Often, this suppresses translation and targets the mRNAs for destruction; but some bound mRNAs escape, presumably because other bound proteins interfere with RBP10 action.
Among RBP10 targets are the mRNAs encoding 3 more regulatory proteins: ZC3H20, ZC3H21 and ZC3H22. Each of these mRNAs has a 3'-UTR of several kb, with 2, 5 and 7 RBP10-binding sites, respectively. ZC3H20 protein expression increases during differentiation to the growth-arrested stumpy form, which is pre-adapted for conversion to the procyclic form, while ZC3H21 and ZC3H22 protein and mRNA appear only in procyclic forms. ZC3H20 and ZC3H21 bind to overlapping, but different sets of mRNAs, and they are together responsible for enhancing expression of some procyclic-specific proteins, especially of the plasma membrane and mitochondrion. ZC3H20 and ZC3H21 act by recruiting a complex that includes MKT1 and PBP1, which in turn recruits PABP (PMID 24470144) and specialized translation factors. Some developmentally regulated procyclic-specific mRNAs can bind both RBP10 and ZC3H20/21, allowing for dynamic regulation during differentiation.

RT.01.2 - **THE INFLUENCE OF RECOMBINATIONAL PROCESSES TO INDUCE DORMANCY IN *TRYPANOSOMA CRUZI***

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The protozoan *Trypanosoma cruzi* causes Chagas disease, a neglected tropical disease that affects 8 million people worldwide. Chagas disease can be divided into two stages: an acute phase presenting high parasitemia and a chronic one of low parasitemia. Recently, the importance of dormancy to drug resistance in amastigote forms of *T. cruzi* have been shown. Here we quantify dormant parasites of different *T. cruzi* DTUs in its replicative forms (epimastigote and amastigote). For this study, cells of *T. cruzi* CL Brener, Y, Dm28c, Bug 2149 and JG strains were used. In order to follow parasite replicative profile in culture, epimastigote forms of the parasites were treated with CellTrace CFSE and incubated in LIT media. Samples were collected every 24 hours, and analyzed by flow cytometry. Parasites showing maximum fluorescence after 96, 120 and 144h of culture growth were considered dormant. For dormancy quantification in amastigote form, trypomastigote parasites of the specific lineages were treated with CellTrace CFSE and incubated for 1h with LLCMK2 cells in DMEM containing 2% of FBS. The total number of amastigotes were determined and parasite still well stained for CFSE 72h and 96h post infection were considered dormant. Hybrid strains, such as

T. cruzi CL Brener (DTU TcVI) and Bug (DTU TcV), showed higher number of dormant parasites when compared to the non-hybrid strains (TcI, TcII), for both epimastigote and amastigote form. Since we have recently shown that hybrid strains present high expression of RAD51, a key gene in recombination process, we also decided to test whether recombination would affect the levels of dormancy. For this we measured the number of dormant cells in T cruzi CL Brener Rad51 single knockout cell. Our results showed a considerable reduction in dormancy in this strain. To confirm the involvement of recombination in generation of dormant cells, we treated *T. cruzi* cells with gamma irradiation that generated DNA lesions, which are repaired by recombination. We observed that gamma irradiation increases the dormancy profile. All together these data indicate that *T. cruzi* DTUs have different ability to generate dormant cells and that recombination process may be important for cell dormancy in this parasite. **Keywords:** Recombination; dormancy; dna repair

RT.01.3 - **CONSERVATION AND FUNCTION OF THE ATR PATHWAY IN *LEISHMANIA MAJOR***

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The remarkable genome plasticity of *Leishmania* limits not only the use of genetic manipulation approaches, but also the establishment of effective leishmaniasis chemotherapy. The strategies used by this protozoan to maintain its genome while allowing variability are not fully understood. With that in mind, we set out to investigate the conservation of key factors of the ATR pathway, a eukaryotic chain of events underlying genome maintenance. To this end, we focused on 3 factors of the pathway: the single-stranded DNA (ssDNA) binding protein RPA1, the checkpoint protein HUS1, and the protein kinase ATR. We have used CRISPR-cas9 editing to endogenously tag and/or delete these factors and using ChIP-Seq we showed that RPA1 and HUS1 were enriched at intergenic and putative centromere regions of the genome in response to replication stress. It is noteworthy that, upon acute hydroxyurea-mediated stress, RPA1 was substantially enriched at the single major early-S replication origin of each chromosome of the parasite. Our findings revealed that HUS1 is essential and is required for a G2/M checkpoint. HUS1 has a conserved role, by which it preserves genome stability and also a divergent role, by which it promotes genome variability. These distinct roles correlate to distinct patterns of formation and resolution of ssDNA and phosphorylated histone H2A throughout the cell cycle, suggesting that *Leishmania* HUS1 has evolved to co-opt canonical genomic maintenance and genomic variation functions. We have also explored the expression and function of the parasite ATR homolog locus. We found that ATR is essential and ATR deficiency significantly affected the response to replication stress. Our results revealed that ATR activity is involved in the correct recruitment or stabilization of RPA1 to ssDNA generated upon replication stress. Altogether, our studies are unveiling pivotal functions of the *Leishmania* ATR pathway in balancing genome stability and transmission. **Supported by:**FAPESP
Keywords: Leishmania; replication stress; atr pathway

RT.01.4 - **THE APPLICATION OF NOVEL GENETIC AND IMAGING TECHNOLOGY REVEALS THAT BENZNIDAZOLE UPTAKE IN *TRYPANOSOMA CRUZI* IS MEDIATED BY ENDOCYTOSIS**

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For almost 50 years, the nitroheterocyclic agent benznidazole has been the front-line treatment for T. cruzi infections. Benznidazole is a pro-drug that is activated within the parasite by the bacterial-like mitochondrial-localised type I nitroreductase TcNTR-1. Reductive metabolism results in the formation of reactive intermediates, ultimately leading to the generation of glyoxal, a mutagen with DNA-glycating and cross-linking activity. Laboratory-induced resistance to benznidazole is readily achievable and has been linked with acquired mutations within the TcNTR-1 gene, or to a reduction in copy-number. Investigations into the mechanisms of benznidazole-resistance have been restricted

by the limited flexibility of T. cruzi genetic tools and the absence of the genetic machinery for RNA interference (RNAi). Because trypanosomatids share many metabolic processes, Trypanosoma brucei RIT-seq genome-wide screening technology can be exploited as a tool to provide insight into drug activity in other parasite species where shared pathways/targets/transporters are involved.
When we used a RIT-seq screen to identify T. brucei genes linked with benznidazole-resistance acquired through loss-of-function, we detected several genes encoding subunits of the vacuolar-type proton ATPase (V-type ATPase), a membrane-localised complex that mediates acidification of intracellular vacuoles, including lysosomes and acidocalcisomes. This enrichment of RNAi target fragments corresponding to V-type ATPase subunits suggested a role for the endocytic pathway in drug uptake. To validate this in T. cruzi, we used a streamlined CRISPR/Cas9 system to generate a range of V-type ATPase subunit single KO and null mutants. These each displayed benznidazole-resistance, implying a common uptake mechanism.
To investigate this further, we chemically linked benznidazole to BODIPY (boron-dipyrromethene) and incubated the fluorescently tagged drug with parasites. Uptake via the flagellar pocket was readily detectable in real-time, followed by transit through the endosomal pathway. Benznidazole sensitivity of T. cruzi in the presence of the specific V-type ATPase inhibitor bafilomycin was also evaluated. This revealed inhibitor antagonism, demonstrating an association between inhibition of complex activity and reduced sensitivity to benznidazole. Therefore, both genetic and chemical validation experiments confirm a role for the V-type ATPase in benznidazole mode of action in T. cruzi.
Progress in dissecting the mechanisms of benznidazole action, both in vivo and in vitro, has been facilitated by advances in transfection technology and imaging procedures. These will be described, and their further applications discussed. **Supported by:**Wellcome Trust
Keywords: Crispr/cas9; benznidazole; trypanosoma

RT.02 – TOXOPLASMA GONDII BIOLOGY AND HOST INTERACTION

RT.02.1 - **MICROVASCULAR DYSFUNCTION AND NEUROINFLAMMATION INDUCED BY *TOXOPLASMA GONDII* IN MICE**

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Toxoplasmosis is one of the leading parasitic diseases worldwide. Some data suggest that chronic acquired toxoplasmosis could be linked to behavioral alterations in humans. The parasite infects neurons, forming immunologically silent cysts. Cerebral microcirculation homeostasis is determinant to brain functions, and pathological states can alter capillarity or blood perfusion, leading to neurodegeneration and cognitive deficits. Albino mice were infected with *T. gondii* (ME49 strain) and analyzed after 10, 40 and 180 days. Infected mice presented decreased CBF as shown by Laser Speckle. Intravital microscopy demonstrated that infection led to significant capillary rarefaction, accompanied by neuroinflammation, with microglial activation and increased numbers of rolling and adherent leukocytes to the wall of cerebral capillaries. Acetylcholine-induced vasodilation was altered at all time points and blood-brain barrier (BBB) permeability was evident in infected animals at 40 dpi. Infection reduced angiogenesis, with decreased number of Isolectin B4-stained blood vessels and a decrease in length and branching of laminin-stained capillaries. Sulfadiazine reduced parasite load and partially repaired microvascular damages. Because of these promising results, we performed in vitro infection of brain microvascular endothelial cells (bEnd.3 cells) with tachyzoites of *T. gondii* (ME49). Infected cultures had reduced tight junction ZO-1 protein immunoreactivity. Accordingly, transendothelial electrical resistance was reduced in infected cultures. Mitochondrial physiology in T.

gondii-infected cells is also currently being investigated by our group. We conclude that *T. gondii* latent infection causes a harmful insult in the brain, promoting neuroinflammation and microcirculatory dysfunction in the brain, with decreased angiogenesis and can contribute to a neurodegenerative process. **Supported by:**CNPq (Edital Universal), PAPES VII/Fiocruz
Keywords: Congenital toxoplasmosis; cerebral microcirculation; neuroinflammation

RT.02.2 - **THE HIGHLY-UNUSUAL YET EVOLUTIONARILY CONSERVED FRAGMENTED MITOCHONDRIAL GENOME OF THE COCCIDIAN, *TOXOPLASMA GONDII***

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The mitochondrial genome sequence, mtDNA, of examined apicomplexan protist parasites is highly-reduced, ~6 kb in length and consists of only three protein-encoding genes and highly-fragmented large and small subunit ribosomal genes. Despite apparent size and gene content conservation, the structures of apicomplexan mtDNA identified to date have been variable, consisting mostly of linear or linear-concatemer arrangements. *Toxoplasma gondii* and the related parasites *Neospora caninum* and *Hammondia Hammondi* do not follow this paradigm. Three fundamental differences are observed. First, the mtDNA of *Toxoplasma* and *Neospora* are physically constructed of 21 distinct sequence blocks, none of which encodes a full gene. Second, the architecture (order, orientation and number) of the 21 sequence blocks is highly-variable. Third, some arrangements of sequence blocks yield contiguous sequence capable of encoding one or more of the cytochrome genes, *cob*, *coxI* and *coxIII*. The three protein encoding genes are observed to be non-redundantly fragmented onto 13 of the 21 sequence blocks. The sequence blocks appear to be joined without the addition or removal of sequence. Nearly all mtDNA molecules sequenced thus far with single-molecule Oxford Nanopore technology are unique with respect to block number, order and orientation. The mtDNA molecules range in length from 2-23.3 kb. Full cytochrome transcripts are detected. Analysis of an ENU cob point mutation revealed that homoplasmy is maintained. The tertiary structure of the genome sequence(s) and mechanism of replication remain elusive. Tissue coccidia are important pathogens of man and animals and the mitochondrion represents an important drug target. The sequence of the mitochondrial genome has remained elusive until now. **Supported by:**National Institutes of Health
Keywords: Mtdna; coccidia; evolution

RT.02.3 - **REGULATION OF MITOCHODRIAL MORPHOLOGY IN TOXOPLAMA GONDII**

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Toxoplasma's singular mitochondrion is very dynamic and undergoes morphological changes throughout the parasite's life cycle. While intracellular, the mitochondrion is maintained in a lasso shape that stretches around the parasite periphery and is in close proximity to the pellicle, suggesting the presence of membrane contact sites. Upon egress, these contact sites disappear, and the mitochondrion retracts and collapses towards the apical end of the parasite. Once reinvaded, the lasso shape is quickly reformed, indicating that dynamic membrane contact sites regulate the positioning of the mitochondrion. We discovered a novel protein (TgGT1_265180) that associates with the mitochondrion via interactions with the fission related protein Fis1. Knockout of TgGT1_265180, which we have dubbed Fip1 for Fission 1 Interacting Protein 1, results in a complete disruption of the normal mitochondrial morphology. In intracellular Fip1 knockout parasites the mitochondrial lasso shape is disrupted, and instead it is collapsed as normally only seen in extracellular parasites. Additionally, proper mitochondrial segregation is disrupted, resulting in parasites with no mitochondrion and extra mitochondrial material outside of the parasites. These gross morphological changes are associated with a significant reduction of parasite propagation and

can be rescued by reintroduction of a wildtype copy of Fip1. We hypothesize that Fip1 mediates contact between the mitochondrion and the pellicle in a regulatable fashion, and that the Fip1-dependent morphodynamics are critical for parasite propagation. Current studies are focused on characterizing the consequences of mitochondrial collapse and identifying proteins that interact with Fip1 to position the mitochondrion to the periphery of the parasite
Keywords: Toxoplasma; mitochondria; membrane contact sites

RT.02.4 - **INVESTIGATING THE CONTRIBUTION OF THE APICOPLAST TO TOXOPLASMA SURVIVAL AND PERSISTENCE AS A LATENT STAGE**

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Acute toxoplasmosis is associated with the rapid replication and spread of Toxoplasma gondii tachyzoites within the body. This infection phase is usually contained by the immune system in immunocompetent individuals. However, the parasites can differentiate into slowly growing bradyzoite forms, establishing within tissue cysts, primarily in the central nervous system and muscle. This persistent chronic form of the pathogen remains in the host throughout its life and can lead to a severe pathology in the event of a weakened immune system.
T. gondii harbors an organelle called the apicoplast, derived from a secondary endosymbiotic event. This plastid contains several metabolic pathways, some of which are essential to tachyzoites. We are investigating the contribution of the organelle to the survival of chronic stage, which is currently incurable.
We are using stage-specific promoters to generate conditional bradyzoites mutants of essential apicoplast genes. We are currently evaluating the impact of the loss of the organelle on the differentiation process of the parasites into the chronic latent form, as well as the survival of fully differentiated bradyzoites, both in vivo and in vitro.
If we manage to generate apicoplast-deficient bradyzoites that are viable but fail to reactivate in vivo, this offers the possibility of using these avirulent parasites as potential vaccines, particularly in livestock to reduce zoonotic transmission. On the other hand, if the apicoplast is essential for the viability of bradyzoites, it would validate the organelle as a source for potential drug targets for this particular parasite stage. **Supported by:**Agence Nationale de la Recherche
Keywords: Toxoplasma; apicoplast; chronic toxoplasmosis

RT.03 – LEISHMANIA SPP AND TRYPANOSOMA CRUZI: SOME ASPECTS OF THE HOST-PARASITE RELATIONSHIP

RT.03.1 - **ROS AND TRYPANOSOMA CRUZI: FUEL TO INFECTION, POISON TO THE HEART**

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Trypanosoma cruzi is one of the few pathogens able to infect heart tissue. Chronic chagasic cardiomyopathy (CCC) develops years after acute infection by T. cruzi and does not improve after trypanocidal therapy despite reduction of parasite burden. During disease, the heart responds to inflammatory signals, such as TNF and IL-1β, and undergoes oxidative stress, potential causative factors for arrhythmias and contractile dysfunction. Our main goal is to determine whether antioxidants and antagonists of inflammatory cytokines can improve cardiac function in CCC and whether these treatments are able to alter parasite burden. Antioxidants such as resveratrol, tempol, and metformin are able to reverse most of the cardiac dysfunction in CCC caused by Colombian strain. Now we are studying the effects of IL-1β, an arrhythmogenic cytokine, as well as the effects of Nrf2 induction and

mitochondrial oxidative stress in CCC. Infected NLRP3-/- were found to have as high levels of IL-1 β during chronic stage of Colombian strain heart disease as wild-type controls, as well as an equally severe heart electrical and mechanical dysfunction. Antagonization of IL-1 β with anakinra or genetic deficiency in IL-1R did not reverse heart dysfunction. The Nrf2-activator CoPP, which we have previously shown to reduce infection/ heart damage during acute disease with Y strain, was not able to improve heart function or avoid its degeneration during chronic Colombian or Y strain infection; on the contrary, it even sharped dysfunction and increased parasite burden. When CoPP was used during the early acute disease of Y strain disease, it reduced parasite burden and improved the outcome at the chronic stage. Antioxidants were able to reduce Y or Colombian strain burden in cardiomyoblasts, while prooxidants increase it. Together, our results are shedding light on important questions that represent starting points to innovative therapies aimed at developing specific treatments to CCC. **Supported by:**CNPq, CAPES, FAPERJ **Keywords:** Trypanosoma cruzi; heart; parasitism

RT.03.2 - **INFLAMMATORY PATHWAYS IN THE VECTOR-LEISHMANIA-HOST INTERPLAY: THE ROLE OF HEME-OXYGENASE**

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Visceral leishmaniasis (VL) remains a major public health problem worldwide. It is important to identify major factors driving the successful establishment of the Leishmania infection to develop better tools for the disease control. Heme oxygenase-1 (HO-1) is a key enzyme triggered by cellular stress. Our group has shown in vitro that HO-1 favors Leishmania infantum, the causative agent of VL cases in Brazil and transmitted by Lutzomyia longipalpis, survival in infected cells by reducing inflammatory responses. Upon L. infantum infection, macrophages from Hmox1 knockout mice presented significantly lower parasite loads when compared with those from wild-type mice. Sand flies bite mammalian hosts to obtain a blood meal, driving changes in the host inflammatory response that support the establishment of Leishmania infection. In the context of Lutzomyia longipalpis-host cell interplay, we showed that exposure to sand fly bites is associated with induction of HO-1 in vivo. This effect is partially attributed to components of sand fly saliva, which are able to recruit and activate leukocytes. Finally, patients with VL presented higher systemic concentrations of HO-1 than healthy individuals, and this increase of HO-1 was reduced after antileishmanial treatment, suggesting that HO-1 is associated with disease susceptibility. Collectively, our data demonstrates that (i) vector saliva induces early HO-1 production at the bite sites, representing a major event associated with establishment of naturally-transmitted Leishmania infections and (ii) that HO-1 has a critical role in the L. infantum infection and is strongly associated with the inflammatory imbalance during VL.
Supported by:CNPQ **Keywords:** Leishmania; heme oxyganese; visceral leishmaniasis

RT.03.3 - ***LEISHMANIA* PARASITES TAKE ADVANTAGE OF FRONT LINE INNATE IMMUNITY TO ESTABLISH A LONG-LASTING INFECTION IN MAMMALS**

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Leishmaniases are a group of diseases caused by *Leishmania* parasites inoculated in mammal skin during the feeding of females of the genus *Phlebotomus* and *Lutzomyia* in the Old and New World, respectively. Macrophages (MØ) are responsible for ensuring a long-lasting *Leishmania* infection, while polymorphonuclear neutrophils (PMN), which are highly armed cells and a key component of

the first line of mammal immune defense against pathogens, efficiently engulf and inactivate *Leishmania* promastigotes. Nevertheless, some intracellular parasites survive and can replicate, contributing to the establishment of *Leishmania* infection. An overview of the impact of PMN intracellular and extracellular effector mechanisms that become activated in response to species of *Leishmania* causing American cutaneous leishmaniasis and zoonotic visceral leishmaniasis is presented, as well as the pathways used by the parasite to be transferred from infected PMN to MØ. The mechanisms involved in PMN activation are discussed, including the gene expression of pattern recognition receptors. Broadening the knowledge of the early interaction of *Leishmania* parasites with mammal innate immunity can shed light in the design of therapeutic and prophylactic tools projected to control the initial infection, which can reduce psychosocial and stigmatizing effects of disease and lead to a noticeable reduction of leishmaniasis. **Supported by:** This study was supported by the Portuguese Foundation for Science and Technology through projects PTDC/CVT-CVT/28908/2017, PTDC/CVT/113121/2009, GHTM–UID/Multi/04413/2013, and CIISA-UID/CVT/00276/2019
Keywords: Innate immunity; leishmania spp.; phagocytes

RT.03.4 - **EXTRACELLULAR VESICLES RELEASED BY LEISHMANIA AMAZONENSIS AND BY B-1 CELLS: IMPACT ON IMMUNITY AND CUTANEOUS LEISHMANIASIS PROGRESSION**

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Leishmania spp are capable of releasing extracellular vesicles (EVs) that contain antigens, virulence factors, RNA, DNA, and lipids. These EVs play an important role in parasite-host relationship, pathogen survival, infection establishment, immunomodulation and adaptation of the parasite to the host environment. Cells infected with Leishmania also release EVs with immunomodulatory properties. Bone marrow derived macrophages (BMDMs) stimulated with *L. amazonensis*-EVs showed an increase in IL-10 and IL-6 expression. We observed a significant higher parasite load and a polarization to Th2 response in BALB/c mice infected with *L. amazonensis* promastigotes in the presence of *L. amazonensis*-EVs, as compared to the group infected with the parasite alone. However, lower parasite burden were detected in animals immunized with EVs, demonstrating an potential use of these EVs in protection models. EVs released by immune cells have emerged as a new important entities in cell-cell communication since their can activate and/or modulate other immune cells. Our group showed that B-1 cells, a subtype of B lymphocytes, spontaneously release EVs, but in the presence of *L. amazonensis* promastigotes a significant increase in EVs production were detected. BMDMs showed an increase in IL-6 and IL-10 expression after stimulating with EVs from infected B-1 cells, as compared to the macrophages treated with EVs from uninfected B-1 cells. In vivo studies showed that mice previously treated with EVs from B-1 cells showed a lower lesion size and a reduction in the parasite load at 7 weeks post-infection with *L. amazonensis*. Thus, our results have contributed to a better understand of the parasite-host relationship and to provide support for the development of new strategies and approachs to be applied in leishmaniasis prevention and treatment. **Supported by:**FAPESP, CNPq, CAPES **Keywords:** Extracellular vesicles; b-1 cells; leishmania amazonensis

RT.04 – CELL BIOLOGY OF TRYPANOSOMES

RT.04.1 - **NUCLEOCYTOPLASMIC TRANSPORT: SEARCHING FOR VULNERABILITES IN TRYPANOSOMES**

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Organisms adapt to changing conditions to survive, and for protozoan parasites this includes host transitions and life cycle progression, mainly achieved through altered gene expression. Nuclear mRNA export is an important component of this process, but poorly understood in trypanosomes where transcription is polycistronic and mRNA is processed by trans-splicing. Orthologs of few proteins involved in mRNA export in higher eukaryotes are detectable. We previously described conserved components of the mRNA export pathway in T. cruzi, including TcSub2, a component of the TREX complex and Hel45, a shuttling RNA helicase. Here we analysed the interactomes of TcSub2 and Hel45 to uncover additional components of this system. We found significant overlap between TcSub2 and Hel45 interactions, suggesting that these two proteins associate with similar machinery. Several TcSub2/Hel45-associated proteins are specific to trypanosomatids. Amongst this cohort were proteins designated by TcFOP-like, TcAPI5-like, TcNTF2-like, and core components of the EJC. We suggest that these lineage-specific innovations involved in mRNA export are likely part of the evolutionary adaptation to polycistronic transcription/trans-splicing. **Supported by:**Fundação Araucária; CAPES; FIOCRUZ-PAPES **Keywords:** Rna transport ; trypanosomes; gene expression regulation

RT.04.2 - **NEW INHIBITORS OF METABOLITE TRANSPORTERS EXHIBIT ANTI-TRYPANOSOMA CRUZI ACTIVITY**

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Polyamines are aliphatic polycations that participate in cell growth and differentiation. In Trypanosoma cruzi the transport of polyamines from the extracellular medium is an essential process because this organism is unable to synthesize de novo these compounds. In a similar way, the amino acid proline is also involved in differentiation processes, cellular invasion and resistance to oxidative, nutritional and osmotic stress. In our laboratory we previously identified in the genome of T. cruzi a multigene family of amino acid and polyamine transporters called TcAAAP (Amino Acid/Auxin Permeases). Two members of this family, the putrescine/spermidine and proline permeases, were functionally characterized and studied as drug targets. In order to identify inhibitors of these permeases, computational simulations combined with in vitro assays were applied. In the case of the proline permease, for the similarity-based virtual screening, the compound crystal violet was selected as a starting point. Crystal violet was used in blood banks as a trypanocidal agent (discontinued due to its high toxicity) whose mechanism of action involves the inhibition of proline transport. To search for polyamine permease inhibitors, the reference molecule was an experimental oncological drug formed by a conjugate of a polyamine (putrescine) with anthracene called ANT4 that acts as a potent inhibitor of the polyamine transport in mammalian cells. Using these compounds, a similarity screening was performed on structures databases of approved compounds to use in humans using molecule shape and electrostatic potential comparison algorithms. Three drugs (loratadine, cyproheptadine and clofazimine) were found to be inhibitors of the proline permease in vitro and also had trypanocidal activity with IC50 between 1 and 13 µM in trypomastigote and amastigote forms. In the case of the polyamine transporter, other three drugs (promazine, chlorpromazine and clomipramine) showed to be transport inhibitors and trypanocidal agents with IC50 between 1 and 4 µM. The strategy herein applied, based on the screening of approved compounds used to treat other pathologies, is known as drug repurposing or drug repositioning. One of the main advantages of this experimental approach is

that reduces the time and the economic cost of implementation of new therapeutic alternatives, which is especially important in neglected diseases, like Chagas. **Supported by:**ANPCyT - CONICET - Global Challenges Research Fund **Keywords:** Trypanosoma cruzi; drug repositioning; metabolite transporters

RT.04.3 - ***TRYPANOSOMA CRUZI* AMASTIGOTES: YOU LIVE BETTER IF YOU KNOW WHEN TO CLOSE YOUR MOUTH!**

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Trypanosoma cruzi proliferative forms must have an efficient endocytic pathway to sustain their speeded up metabolism. The unique cytoskeleton constituted by a corset of microtubules underneath the plasma membrane restricts the formation of endocytic vesicles to the membrane of the cytopharynx. Amastigotes obtain their nutrients from the host cell cytosol, so they have access to macromolecules that had already been processed by the host cell endocytic machinery or were synthesized by that cell. It is interesting to investigate how they obtain iron or cholesterol, two essential inputs they will need in order to build their own membranes and respiratory chains. There are few studies about amastigote endocytic features, certainly due to the difficulty to place a tagged endocytic tracer in the cell cytoplasm. On the other hand, experiments that use endocytic protocols similar to those standardized to epimastigotes, just substituting them by amastigotes that had been extracted from host cell or obtained from the supernatant of infected cultures, did not succeed. In this talk we will compare the endocytic ability of T. cruzi epimastigotes, extracellular and intracellular amastigotes and present explanations for this discrepancy.

RT.04.4 - **HOW TRYPANOSOMA CRUZI OT TRYPOMASTIGOTES ESCAPE FROM INFECTED CELLS**

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Over the years, several studies addressed cell invasion by T. cruzi but less is known about its egress. Some researches demonstrate that, similar to other intracellular pathogens, disruptions occur in the three cytoskeleton-forming filaments during intracellular parasite development. Others suggest that in the late stages of infection, calcium influx may occur due to increased cell permeability, promoting release of parasite proteases. Literature also describes that adherent infected cells releases trypomastigotes through ruptures at the edges; however, rounded cells were also observed prior to parasite egress. Here we show aspects of T. cruzi egress using confocal live cell imaging and correlative images techniques (confocal observations and later visualization on scanning electron microscopy (SEM)).
Supported by:FAPESP **Keywords:** Trypanosoma cruzi; trypomastigote; egress