PV-13 - **The expression of positive and negative mutants of TcRlp, a Ras-like GTPase of *Trypanosoma cruzi,* suggests a role for this protein in epimastigote proliferation and metacyclogenesis**

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Ras family GTPases act as molecular regulators of essential cellular processes, such as cell proliferation and differentiation, alternating between active (GTP-bound) and inactive (GDP-bound) stages. These GTPases are also modified by isoprenylation (geranylgeranylation or farnesylation) in a CaaX motif that allows association with membranes. The parasite *Trypanosoma cruzi* has only one Ras family gene, which encodes the protein TcRlp and has a CaaX CVLL motif, which is geranylgeranylated. Previously, the TcRlp gene was cloned from the *T. cruzi* (Dm28c) and several mutant versions were produced, including: TcRlp-Q61K (positive dominant), TcRlp-S17N (negative dominant) and TcRlp-CQLF (farnesylated mutant). The wild-type TcRlp gene and its mutants were cloned into the pTEX-GFP plasmid, allowing for their expression with a GFP tag. This work investigates the impact of TcRlp mutants in *T. cruzi* biology, assessing cell localization of mutant proteins by fluorescence microscopy, evaluating the epimastigote proliferation potential through growth curves on LIT and assessing metacyclogenesis capacity with the TAU-3AAG assay. The microscopy analysis reveals that the TcRlp-S17N and -CQLF are mostly localized in the nucleus, while TcRlp-WT the -Q61K display predominant cytoplasmic localization. The growth curve revealed that cells expressing TcRlp-Q61K present a lower growth, when compared to GFP control, in the presence of either 1 or 10% FBS, while TcRlp-S17N displays higher proliferation, which is more apparent in 1% SFB. Analysis of trypomastigote/epimastigote proportions at 48 and 96 hours of TAU-3AAG stimuli revealed that metacyclogenesis increases in cells expressing either the TcRlp-S17N or -Q61K mutants, and that the proportion of metacyclics to epimastigotes for -Q61K is ~1.5 times higher than the observed for -S17N. The results obtained in this study points toward a role for TcRlp as a putative negative regulator of cell grown and positive promotor of metacyclogenesis.
Keywords: Ras family GTPases;Trypanosoma cruzi;Metacyclogenesis.

PV-14 - **Evidencing centromeric regions in Trypanosoma cruzi: an in silico and experimental approach**

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Trypanosomatids have a peculiar genome organization and transcription is arranged in polycistronic transcription units (PTU). Mitosis in T. cruzi occurs with chromosome semi-condensation, hampering classical cytogenetic approaches. Few studies explored the relationship between centromeric regions and kinetochore proteins governing chromosomal segregation. Centromeres have been studied through high throughput (Hi-C) analysis. Hi-C data reveals a genome-wide DNA-DNA contact matrix, enabling PCA analysis for centromeric region identification. Investigating T.cruzi Brazil A4 Hi-C data (Wang et al 2021), we highlighted 11 putative centromeric regions. Canonical eukaryotic kinetochore proteins have not been identified in trypanosomatids, but 25 kinetoplastid kinetochore proteins (KKT) were described in T.brucei (Akiyoshi B, Gull K. 2014). We focus on characterizing centromeres using PCA patterns and two KKTs constitutive of centromeric DNA. In silico investigation involves BLASTn searches against centromeric DNA of T. cruzi CL brener, synteny analysis, and genomic features (PTU directions and GC content). These strategies identify the whole centromeric region of T. cruzi CL Brener chromosome 3 on chromosome 20 of T. cruzi Brazil A4. We also found the typical PCA pattern from Hi-C analysis, representing DNA-DNA interactions at the chromosome arms. We will validate T. cruzi putative centromeric regions by generating labeled T. cruzi strains for two KKT proteins and determining their genomic location by Chip-seq. In summary, we expect to confirm Hi-C's ability to inform centromere location in T. cruzi through experimental analysis, enhancing our understanding of genome organization in trypanosomes. **Supported by:**FAPESP-Processo 2022/12897-4 **Keywords:**Centromere ;Kinetochore;Hi-C.

PV-15 - **Isolation of active ribosomes by RiboLace enables the study of *Leishmania* translational adaptation**

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Protozoan parasites of the genus *Leishmania* exhibit great phenotypic variability in terms of clinical symptoms, illness prognosis, and treatment susceptibility, complicating disease management. Such variety is impressive considering the parasites' constitutive gene expression, which lacks traditional promoter-driven regulation, raising questions on how *Leishmania* adapts and develops in response to environmental change. Using an experimental evolution approach, we have discovered various regulatory mechanisms that govern *Leishmania* fitness gain in culture, including gene dosage changes caused by genomic instability, and compensatory responses at post-transcriptional and translational levels. These findings point to the presence of fitness-adapted ribosomes, which may aid parasite adaptation by filtering out the effects of deleterious gene dosage effects while promoting the effects of beneficial ones. Here we use RiboLace (Immagina Biotechnology) to specifically profile only active ribosomes to investigate adaptive changes in mRNA translatability. We initially tested the method's quantitative application by creating *L. donovani* transgenic parasites that express different levels of GFP caused by modifications in the Kozak sequences (CTTTA: intermediate expression, LdGFP_I; CCACC: high expression, LdGFP_H) and the insertion of hairpins at the 5'UTR (low expression, LdGFP_L), both of which impact mRNA/ribosome interaction. Flow cytometry analysis showed a 4-fold reduced mean fluorescence intensity in LdGFP_I parasites compared to LdGFP_H. RiboLace-isolated, active ribosomes from both LdGFP_I and LdGFP_H strains showed a 2.74-fold higher recovery of GFP mRNA in the latter parasites, thus validating the applicability of RiboLace to quantify differences in translatability. We are currently employing this approach to investigate the role of translational control and the existence of fitness-adapted ribosomes in *Leishmania* during adaptation to *in vitro* culture. **Supported by:**This project is supported by a grant from the Institut Pasteur 'Programmes Transversaux de Recherche' (PTR 425-21) and European Research Council (ERC) Synergy grant for the project 'DECOLeishRN – Decoding epistatic genome/RNome interactions in eukaryotic fitness gain using Leishmania parasites as a unique model system', Grant agreement ID: 101071613
Keywords: Leishmania;adaptation;ribosome.

PV-16 - **The dose makes the poison: analyses of the possible cytotoxicity of thymidine analogs used to monitor DNA replication in trypanosomatids**

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Thymidine analogs (5-Bromo-2′-deoxyuridine – BrdU and 5-Ethynyl-2'-deoxyuridine – EdU) are widely used to monitor DNA replication in eukaryotic cells. However, recent articles have been reporting certain levels of cytotoxicity in mammalian cells, which can bias data related to the replication capacity of the analyzed cells. In trypanosomatids, there are no reports of toxic effects related to the use of thymidine analogs. Thus, there are three possibilities: BrdU and EdU are not toxic for these parasites at standard working concentrations; BrdU and EdU are toxic at standard working concentrations and no research group has evaluated this toxicicity; or only one of these analogs is toxic. To find out which of these possibilities is correct, we investigated the possible cytotoxic effects of BrdU and EdU on *Trypanosoma cruzi*, *Trypanosoma brucei*, and *Leishmania major*. Preliminary data suggest that EdU is toxic at working concentrations to *L. major*. Considering that the only difference between EdU and BrdU is the presence of the alkyne group in place of the bromine atom in position 5, this preliminary result justifies the beginning of a further investigation into the cytotoxicity of alkyne groups present in the DNA. Currently, we are treating the trypanosomatids with different concentrations of BrdU and EdU and evaluating their growth curves, concomitantly with cell viability assays. Moreover, we are investigating the presence of possible cell cycle arrests by the analysis of DNA content using flow cytometry. We also intend to investigate the presence of DNA damage and DNA damage response through IFA using α-γH2A, and by TUNEL assay. If we find DNA damage, we intend to track the regions of the genome where damage is occurring by ChIP-seq using α-γH2A. Of note, a better understanding of the essential molecular mechanisms during the life cycle of these organisms can contribute to the development of specific strategies to combat the diseases caused by these parasites. **Supported by:**Higher Education Improvement Coordination (CAPES), and São Paulo Research Foundation (FAPESP) **Keywords:**BrdU and EdU;DNA replication and damage;Trypanosomatids.

PV-17 - **THE ROLE OF TCCSB IN MITOCHONDRIAL DNA REPAIR ASSOCIATED WITH TRANSCRIPTION IN *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi, the cause of Chagas disease, has unique mitochondria. Nucleotide excision repair pathway in this organelle remains uncharacterized. In this study, we investigated the role of CSB, a gene involved in transcription-associated nucleotide excision repair. Cells modified for the CSB gene (single knockout or overexpressing) were exposed to Doxorubicin (targets mitochondria - MtDOX, or nucleus - Dox) and cisplatin (both organelles). After exposure to of MtDox, TcCSB-deficient cells showed increased sensitivity 1h after treatment compared to the wild-type cells, but resumed their growth after 24h. On the other hand, TcCSB overexpressing cells exhibited increased resistance 1h after treatment compared to WT cells, and their growth was halted. After treatment with of Dox we observed that the TcCSB-overexpressing cells were more sensitive, and the TcCSB-deficient mutant was more resistant 1h after treatment, although the former resumed their growth more quickly. When treated with of cisplatin, the same response as with Dox was observed. We then analyzed the repair kinetics with cisplatin and found that the TcCSB-overexpressing cells repaired the mitochondrial damage more rapidly, while the TcCSB-deficient cells had slower repair. We also evaluated changes in mitochondrial transcription after cisplatin treatment and observed that TcCSB-overexpressing cells showed a decrease in transcripts immediately after treatment. After 1h of treatment, the overexpressing cells had already recovered the transcript levels, while the levels continued to decline in the other strains, with a more pronounced decrease in the TcCSB-deficient strains. These results suggest the involvement of TcCSB in mitochondrial repair, and its absence somehow impairs the metabolism of kDNA in *T. cruzi*. The data show that unlike all organisms studied so far, *T. cruzi* has a DNA repair pathway associated with transcription, which may be related to the fact that it has only one mitochondria. **Supported by:**CNPq, FAPEMIG e CAPES
Keywords: Trypanosoma cruzi;kDNA;Mitochondrial DNA repair.

PV-18 - **Iron Overload could Induce Ferroptosis in *Trypanosoma cruzi***

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More than a century after its discovery, Chagas Disease is now considered an emerging disease due to the growing number of cases in non-endemic countries. The etiologic agent *Trypanosoma cruzi* is the object of intense studies worldwide and essential cellular and molecular mechanisms for the parasite's life, as is the case of ionic transport processes. One of these processes is the transport of iron (Fe), considered a critical ionic species in all living organisms, especially those related to energy metabolism, ATP synthesis, DNA synthesis, and the production of reactive O_2 species (ROS). Due to its shallow redox potential, Fe is both necessary and harmful, "friend and foe," depending on the conditions of its use by the cells. Fe overload could induce lipid peroxidation, inducing an iron-dependent cell death called Ferroptosis. Recently, we described an ionic Fe transport mechanism for *T. cruzi* involving two primary proteins: a Fe-reductase essential for reducing Fe^{2+} of the Fe^{3+} found in the extracellular environment and an iron transporter, internalizing Fe^{2+} as substrate. In addition, we described that exogenous Fe is required for proper signaling to control parasite proliferation and H_2O_2 formation, which stimulate parasite differentiation, thus interfering with parasite virulence, comparing parasites submitted to Fe depletion. We aim to evaluate the effect of exogenous Fe overload on the proliferation of epimastigotes of *T. cruzi*, on the regulation of ROS production, and the functional state of antioxidant enzymes, focusing on the possible involvement of the parasite's mitochondria in these processes and the role of Fe in ATP synthesis. Fe overload leads to the arrest of epimastigotes proliferation, and this effect is reverted using Ferrostatin-1, a potent inhibitor of ferroptosis. These results might indicate that Fe overload induces cell death in *T. cruzi*, probably via ferroptosis. **Keywords:**Ferroptosis;parasite lipid content;parasite oxidative stress.

PV-19 - **Investigation of the potential SUMOylation of RPA and its involvement in the nuclear export of this protein in infective life stages of *Trypanosoma cruzi***

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Chagas disease is a prevalent health issue in Latin America and it is crucial to understand the biology of its etiological agent, *Trypanosoma cruzi*. During its life cycle, the parasite alternates between replicative and infective forms and a key component in this context is Replication Protein A (RPA), which plays a vital role in genomic stability and DNA replication. *T. cruzi* relies on three RPA subunits and our previous work highlighted that RPA-1 and RPA-2 are exported from the nucleus to the cytoplasm in its infective life stages, and blocking this export hampers the differentiation. However, the molecular mechanisms underlying this export remain poorly understood. This study aims to shed light on them.
Through *in silico* assays, we have developed three-dimensional models of the RPA complex and found a nuclear export signal (NES) in the RPA-2 subunit, implying its potential involvement in RPA export during *T. cruzi* differentiation. Additionally, we have identified potential SUMOylation sites that, through molecular dynamics simulations, we have uncovered to stabilize the protein, leading us to speculate about its potential involvement in the exposure of the NES through conformational changes.
To validate these findings, we are employing a genetically modified bacterial strain capable of producing SUMOylated proteins. This system involves the introduction of plasmids containing the SUMO protein gene from *Trypanosoma brucei* (68% identity with *T. cruzi* SUMO protein) along with enzymes responsible for activating and ligating SUMO to the target protein. By introducing RPA-1, RPA-2, and RPA-3 genes into this system, which are already inserted in separated pET28a+ plasmids, we can co-express them. The potential SUMOylation of RPA will be examined through immunoblotting of lysed cells, where the presence of higher bands will indicate SUMOylation of the protein. **Supported by:**FAPESP - Processo 2021/12872-9 **Keywords:**Trypanosoma cruzi;Replication Protein A;SUMOylation.

PV-20 - **Endosymbiosis in trypanosomatids: the symbiotic bacterium regulates the oxidative metabolism of the host *Angomonas deanei***

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The coevolution of *Angomonas deanei* with a symbiotic bacterium represents an important model for studying the origin of organelles in the eukaryotic cell. In this mutualistic relationship intense metabolic exchanges occur between both partners and the symbiont completes essential biosynthetic pathways of the host protozoan. In this work, wild type (symbiont-harboring cells - AdWt) and aposymbiotic (AdApo) strains of *A. deanei* were compared under different nutritional conditions in order to investigate the influence of the symbiont on the intermediary and energy metabolism of *A. deanei* Data obtained by transmission electron microscopy show that AdWt presents marked ultrastructural alterations when compared to AdApo, especially in the mitochondrion and after cultivation in medium with proline. The O_2 consumption was 30% higher in the AdWt strain than in AdApo when cells were grown in the presence of glucose or proline. Both strains did not show significant variation in ATP levels after growth under such nutritional conditions. Data obtained by proteomic analysis indicate that the enzymes of the Krebs cycle, as well as those of the glycolytic and fermentation pathways are more expressed in AdApo and those of oxidative phosphorylation in AdWt. Taking together, data indicate that the symbiont promotes the recovery of intermediate metabolites, regulating and optimizing the oxidative metabolism of *A. deanei*. **Supported by:**88887.604982/2021-00 **Keywords:**endosymbiosis;intermediate metabolism;energy metabolism.

PV-21 - **Role of phosphorylation of Orc1Cdc6 protein in the cell cycle of *Trypanosoma cruzi***

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The process of DNA replication in the cell cycle requires strict control to ensure genomic stability. The pre-replication complex (PRC) performs this control, being formed, in most eukaryotes, by the Orc hexamer and the proteins Cdc6 and Cdt1, which act together to allow the action of the MCM complex. On the other hand, the replication process of *Trypanosoma cruzi* (*T. cruzi*), the etiologic agent of Chagas disease, is poorly understood. Its life cycle involves replicative (epimastigotes and amastigotes) and non-replicative forms (blood and metacyclic trypomastigotes) and has a protein, named Orc1Cdc6, composing the PRC. Orc1Cdc6 is present in the nuclear space in all stages of the life cycle, but without interacting with DNA in non-replicative forms, raising the hypothesis of possible post-translational modifications (PTMs) involved in the control of the interaction of Orc1Cdc6 with DNA. To investigate this hypothesis, we searched for possible phosphorylation sites in the Orc1Cdc6 protein (TriTrypDB Database code Tc508239.10) on the NetPhos website, resulting in 38 possible sites after data processing. We analyzed the same sequence on the PhosTryp website, which resulted in 8 possible phosphorylation sites in common. We also carried out structural modeling of the protein using the PyMOL software, which showed that 7 of the 8 sites found are exposed in the protein, being susceptible to phosphorylation. For in vitro analysis, parasites of the CL Brener strain with three myc tags was generated (TcOrc1/Cdc6_3myc) and submitted to immunoprecipitation and Phostag-gel assays, in epimastigote and metacyclic forms. Western blotting of the phostag gel revealed a differential migration of the Orc1Cdc6 protein from different morphologies. Thus, our data show that Orc1Cdc6 is differentially phosphorylated during the life cycle of T. cruzi. The biological implications of these modification(s) will be investigated. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Processos 20/00694-6 e 23/00557-7 **Keywords:**Trypanosoma cruzi;Orc1Cdc6;Phosphorylation.

PV-22 - **Unveiling Cellular Structures in Human and Veterinary Pathogens: A Closer Look with Expansion Microscopy**

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The Expansion Microscopy (ExM) technique offers a unique advantage by surpassing the limitations of traditional light microscopy, particularly the diffraction limit of light (~250 nm), leading to great advances in various fields of interest, one such area is the refinement of protein localization in pathogens. Is based in the use of a super hydrophilic polymer that permeates and make crosslinks with the biological sample, resulting in the formation of a swellable gel when in contact with water. The proteins of the sample become anchored to the polymer network due the crosslinks, allowing for an isotropic expansion of the specimen. Our initial focus was on analyzing the cytoskeleton of two human pathogens, *Trichomonas vaginalis* and *Trypanosoma cruzi*, by labeling tubulin in expanded cells. Additionally, we aimed to investigate the unique structure called costa, a prominent striated fiber found in *Tritrichomonas foetus*, a parasite that affects cattle and cats. As a result, we successfully achieved expanded cells with an expansion factor of approximately 4.6x. In all three types of cells, the labeled cytoskeletal protein complexes displayed stretching in all directions without any signs of fragmentation, maintaining the proportional integrity of the parasites. However, it is worth noting that membrane proteins did not exhibit successful labeling after expansion. This aspect necessitates further modifications to the protocol, such as considering an alternative denaturation process that does not compromise these membrane components, such as the plasma membrane. Additionally, this technique can be applied to whole cells and purified fractions for nanostructure research. The stability of the gels allows for their utilization up to 15 days later for additional labeling. Further studies using ExM can help understand the relationship of these protein components with other structures and organelles. **Supported by:**FAPERJ **Keywords:**Expansion microscopy;Cytoskeleton;Fluorescent imaging.

PV-23 - **COPPER CHLORIDE AND SULFATE SOLUTIONS AGAINST EPIMASTIGOTES OF *Trypanosoma cruzi* (CHAGAS, 1909): AN *IN VITRO* PRE-CLINICAL DATA**

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Chagas disease (CD) is caused by the protozoan *Trypanosoma cruzi* affecting 6-7 million people worldwide, mainly in Latin America. Treatment of CD is based on the use of benznidazole and nifurtimox. However, both drugs present low efficacy during the chronic phase of CD, causing severe side effects. Therefore, a high-efficacy less-toxic treatment for CD is urgently needed. Pre- and clinical trials have proven the “biocide” effect of copper, but rarely in *T. cruzi*. This study aimed to test copper sulfate ($CuSO_4$) and copper chloride ($CuCl_2$) against epimastigotes from the Dm28c clone of *T. cruzi*. The parasites (2e6 cells/mL) were incubated with the solutions (Cu-II) in different concentrations (0 – 2000 $\mu mol^{-1}/L$) for nine days at 28°C and counted daily in a Neubauer chamber. Parasites incubated with only LIT medium were considered negative control, and a group treated with paraformaldehyde 4% was considered positive control. The antiparasitic activity was evaluated by the daily replication rate. The data indicate the low-to-high inhibition (concentration-dependent) of parasite proliferation starting from the smallest concentrations of the Cu-II solutions. Both solutions' statistically significant antiproliferative activity was observed at the three highest concentrations ($P < 0.05$). Moreover, the statistical variance was also present between the highest concentrations compared to the lowest ones of both tested solutions. In addition, $CuSO_4$ presented a better effective antiparasitic activity than $CuCl_2$, despite the absence of statistical variance between the groups ($P > 0.05$). Our preliminary data show that Cu-II solutions were active against *T. cruzi* and can be considered a potential new antichagasic therapy. A new round of experiments is planned to provide host cell-safety data. **Keywords:**Metallotherapeutic agents;Antichagasic;Therapy.

PV-24 - **Deciphering the role of Orc1b in the activation of DNA replication in *Trypanosoma cruzi***

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Trypanosoma cruzi genome is high repetitive due the presence of rapidly evolving multigenic families that code for surface proteins that contribute the success of infection. DNA replication is the process that warrant genomic maintenance; however, incomplete, erroneous or premature DNA replication can generate mutations and SNPs, chromosomal aneuploidy or polyploidy, and gene copy number variation. Since the genomic plasticity plays a key role as T. cruzi infection success, it is important to dissect the molecular bases of DNA replication in this parasite. The duplication of the genome beginning in the G1 phase of the cell cycle when the Origin Recognition Complex (ORC), composed in eukaryotes by six subunits, is recruited onto replication origins. The composition of ORC in Trypanosoma, however, is still under discussion. Four proteins were described as ORC components, Orc1Cdc6, 7980, 3120, and ORC4. In Trypanosoma brucei, Orc1b was identified as a Orc1Cdc6 interactor, involved at DNA replication, but that interacts with ORC only during S stage of the cell cycle. Here, we performed the Chip-seq methodology using anti-TY1 in a T. cruzi lineage containing tagged Orc1b. The Orc1b localization was analyzed in relation to previously data from our group that include Orc1Cdc6 localization and active (Orc1Cdc6 sites that was fired) and inactive (not fired Orc1Cdc6 sites) origins. We found that Orc1b co-localizes with Orc1Cdc6. Moreover, while Orc1b in found at Orc1Cdc6 sites and upstream Orc1Cdc6 in inactive origins, it was found only upstream Orc1Cdc6 sites at active ones. These data suggest that Orc1b interacts with ORC even in non-active origins and is dislocated during origin activation. The presence of Orc1b in dormant origins raises the discussion concerning the role of Orc1b in origin activation proposed for T. brucei. therefore we are now investigating the possibility of Orc1b replaces Orc1Cdc6 in some DNA regions. **Supported by:**2020/00694-6 Fapesp **Keywords:**Trypanosoma cruzi;DNA replication;Origin Recognition Complex.

PV-25 - **Iron Storage in *Trypanosoma cruzi***

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Fe is an essential cofactor in many metabolic pathways. The parasite *Trypanosoma cruzi* causes Chagas' Disease, and heme and ionic Fe are required for optimal growth, differentiation, and invasion. Fe is also harmful due to catalyzing the formation of reactive O_2 species; for this reason, all living systems develop mechanisms to control the uptake, metabolism, and storage of Fe. Two main proteins — a Fe-reductase, necessary for reducing Fe^{2+} of the Fe^{3+}, and an iron transporter, which takes up Fe^{2+} as substrate — are involved in the ionic Fe transport through *T. cruzi* plasma membrane. Once in the cytosol, free Fe must be stored or metabolized to avoid the production of free radicals. Bloodstream forms of *T. cruzi* contain iron-rich acidocalcisomes, indicating that this organelle could be responsible for Fe storage. Although free Fe was not observed in acidocalcisomes in other evolutionary forms, this cannot rule out the possibility that in cultured cells, Fe supplementation causes acidocalcisomes of different evolutionary forms to have different functions or storage resources. In plants and *Saccharomyces cerevisiae*, specific membrane proteins transport iron (VIT1/FPN2/Ccc1) into vacuole storage. Here, we found a putative vacuolar iron transporter sequence: it was found in the genome database of *T. cruzi* (TriTrypDB: BCY84_12276) following a BLAST search using the Fe transporter TbVIT from *T. brucei* (TritrypDB: Tb927.4.4960) as a target. This VIT1 ortholog in *T. cruzi*, TcVIT, could be responsible for Fe storage in *T. cruzi*. The deduced amino-acid sequence of the peptide comprises 469 residues, thus resulting in a predicted molecular mass of 50.6 kDa. The deduced protein TcVIT has five possible transmembrane domains and a highly conserved domain corresponding to the vacuolar metal transporter (VIT1/CCC1) family domain. The localization of this vacuolar iron transporter on acidocalcisomes could indicate the role of this organelle in iron storage. **Keywords:** Fe storage;acidocalcisome; trypanosomatids.

PV-26 - **DESIPHERING THE *TRYPANOSOMA CRUZI* HIGH MOBILITY GROUP B PROTEIN (TcHMGB) ROLE IN GENE TRANSCRIPTION**

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High Mobility Group Bs (HMGBs) are abundant non-histone chromatin proteins that contribute to chromatin organization and function, impacting various cellular processes including gene expression, DNA replication, repair, and recombination. Through interactions with DNA and other proteins they can influence the accessibility of genes and the assembly of transcriptional complexes. *Trypanosoma cruzi* HMGB (TcHMGB) is expressed in the nucleus in all the parasite life stages and can alter chromatin structure like its mammalian orthologs. Given the unique characteristics of transcription in trypanosomatids and evidence of epigenetic mechanisms controlling gene expression, our hypothesis is that TcHMGB may have a key role in transcription control.
With the aim of investigating TcHMGB functions, we first constructed transgenic parasites capable of overexpressing the protein under tetracycline induction, which showed that *Tc*HMGB can alter chromatin DNA structure making it more sensible to micrococcal nuclease treatment. *Tc*HMGB overexpression affected the parasite fitness, caused a dramatic decrease in epimastigote- and amastigote-proliferation, presumably impairing cell division and lowered trypomastigote infectivity *in vitro*. Then, we used CRISPR/Cas9 to generate knockout (KO) mutants. We verified gene edition by PCR and lower TcHMGB expression by qRT-PCR and western blot in transient transfectants. However, despite multiple attempts, we were unable to generate stable *TcHMGB* KOs, which suggests TcHMGB gene is essential or crucial for the parasite´s normal functioning.
Finally, to study TcHMGB role on transcription, we induced its overexpresion on epimastigotes and analized the RNA content using different approaches: labeling and detection of nascent RNAs; qRT-PCR and RNA-seq. Our results suggest that although some genes showed differential expression in overexpressing parasites, TcHMGB seems to have more global effects. **Supported by:**AGENCIA I+D+I, PICT 2019-4212;.CONICET, PIP 2021-0848 **Keywords:**TRYPANOSOMA;HIGH MOBILITY GROUP B;TRANSCRIPTION.

PV-27 - **Changes in chromatin accessibility reveal opposing patterns between tRNA genes with most genomic compartments during the *T. cruzi* differentiation.**

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The tRNA genes (tDNAs) contain the genetic information for transfer RNA (tRNAs) expression, which function as an adapter molecule linking a specific amino acid to its corresponding mRNA codon during the protein synthesis. *T. cruzi,* the etiologic agent of Chagas Disease, has a complex life cycle with different life forms living in vertebrate or invertebrate hosts. Recently, we detected by the FAIRE-seq analysis that the open chromatin regions are more enriched in epimastigote (Epi) than in metacyclic trypomastigote (MT) forms mainly at tDNAs. Interestingly, the differentiation of Epi to MT life forms is followed by a decrease in their translatome and transcriptome. We hypothesized that the closed tDNAs chromatin observed in MT might be associated with these events and could play a key role in parasite differentiation. Therefore, this work aimed to analyze whether the tDNA chromatin changes are essential to regulate tRNA expression and to determine the moment, during metacyclogenesis, that tDNA's chromatin became closed. Thus, we applied the FAIRE-seq technique during the epimastigote to metacyclic trypomastigote differentiation. Our results revealed an overall increase in tDNAs open chromatin upon 24 hours of parasite differentiation in TAU 3 AAG medium. In contrast, the open chromatin profile decrease at other loci, such as those from protein-coding genes and interpolicistronic regions. These findings are intriguing and suggest a higher tRNA transcriptional activity in epimastigotes incubated in TAU 3 AAG for 24 hours than in epimastigotes in the exponential phase. Currently, we are performing tRNA-seq and ChIP-seq to respectively measure mature tRNA expression and quantify the presence of RNAP III machinery in the tDNAs during parasite differentiation. These data will be essential for understanding the role of tDNA chromatin alterations and parasite differentiation. **Supported by:**FAPESP 21/11419-9 e FAPESP 18/15553-9 **Keywords:**Trypanosoma cruzi;tDNA;cell differentiation.

PV-28 - **THE PROFILE OF RESEARCH IN TRITRYPS BETWEEN 2010 AND 2021: A SYSTEMATIC REVIEW**

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Leishmaniasis, Chagas disease and sleeping sickness (TriTryp diseases) are caused by the parasites Leishmania spp., *Trypanosoma cruzi* and Trypanosoma brucei, respectively. These infections represent a global public health concern, particularly in tropical regions of South America, Asia and Africa. The aim of our study was to systematically evaluate publications of TriTryp articles over the last 12 years, with a special focus on Brazil, historically a prolific producer of Trytrips articles due to the prevalence of the disease and the studies by Carlos Chagas developed in the early 20th century. Three comprehensive searches were performed on Medline-PubMed to collect publications from January 1, 2010 to December 31, 2021. Using the MeSH terms: leishmania or leishmaniasis; brucei, Trypanosoma or African sleeping sickness; *Trypanosoma cruzi* or Chagas. Data analyzes were performed using the R programming language (version 4.2.1). For the analysis, 6,478 articles were included, with Brazil contributing an average of 27.5% of the publications. Brazilian publications increased from 405 to 726 per year in the last decade. Regional disparities were observed, with the Southeast region leading and the Northeast/North regions with lower averages. The Northeast presented a growing index of publications. Leishmania was the most studied parasite in Brazil. Original articles predominated, mainly in the Southeast region. International collaborations were associated with greater quantity and impact of publications. The Southeast had the highest impact factor, while the Northeast showed significant growth. This study emphasizes Brazil's importance in TriTryp research, highlighting increased publications in the Northeast and North, particularly on leishmaniasis compared to Chagas disease. Regional variations in publication rates, publication type, parasite focus, collaboration, and impact underscore the need for ongoing research to address these public health challenges. **Supported by:**FAPEMIG, CNPq, CAPES. **Keywords:**TriTryps;Manuscripts Evaluation;Regional Development.

PV-29 - **Molecular and structural characterization of *Trypanosoma cruzi* flagellar pocket cytoskeleton**

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Trypanosoma cruzi is a flagellated protozoan of the *Trypanosomatidae* family that displays a **flagellar pocket structure (FP)**, an invagination of the plasma membrane from which the flagellum emerges. Significant studies highlight the FP as an important site of endo/exocytosis, which contributes to the parasite's different survival mechanisms. In *T. brucei* trypomastigotes, several FP structural proteins were described with important function in cell morphogenesis and viability, as TbBILBO1, TbMORN1, TbFPC4 and TbSPEF1. Among these, TbBILBO1 stands out for being essential for FP formation and it was localized exclusively in the FP collar. Genomic data suggested the existence of orthologs for these proteins in *T. cruzi*, in which the FP remodeling is important during differentiation in the three developmental forms: epimastigotes, trypomastigotes and amastigotes. Using CRISPR-Cas9 technique, we generated epimastigote cell lines expressing TcMORN1, TcFPC4 and TcSPEF1 tagged with mNeonGreen (mNG) tag. Immunofluorescence assays using anti-BILBO1 antibody indicated that the protein is not located exclusively in the flagellar pocket collar of *T. cruzi* epimastigotes, but also in a projection that extends into the interior of the cell body. Negative staining of cytoskeleton fractions showed the participation of BILBO1 in the cytostome-cytopharynx, a structure related to endocytic processes in *T. cruzi* epimastigotes. TcBILBO1 exhibited a partial colocalization with TcMORN1, which encircles the flagellum exit site. Our data strongly suggests that BILBO1 and MORN1 organizations are different between *T. brucei* trypomastigotes and *T. cruzi* epimastigotes and this may be related to different morphology and ecological niche. Subcellular and relative localizations and ultrastructural assays are being performed with the other tagged proteins in all forms of *T. cruzi* to better describe the molecular and structural organization of the flagellar pocket. **Supported by:**FAPERJ - 201.908/2022 **Keywords:**Trypanosoma cruzi;flagellar pocket;cytoskeleton.

PV-30 - **CHARACTERIZATION OF CRK1 KINASE IN THE CONTROL OF CELL PROLIFERATION AND METACYCLOGENESIS OF *Trypanosoma cruzi***

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Cyclin-dependent kinases (CDKs) are essential proteins that regulate the cell cycle in eukaryotes, and their modulation depends on environmental and mitogenic factors. In *Trypanosoma cruzi*, the causative agent of Chagas disease, different evolutionary forms can be found in its hosts, differing in terms of morphology, metabolic profile, and replicative capacity. The transition between replicative and infective forms depends on different factors such as nutritional stress and osmolarity changes, which can lead to progression, quiescence, and cellular differentiation. Considering the importance of proliferative control for the life cycle of *T. cruzi*, the present study aimed to investigate and characterize the CRK1 kinase (cdc2 related kinase), a homolog of CDK1, throughout the cell cycle and in the metacyclogenesis of the parasite. To this end, the CRISPR/Cas9 methodology was used to obtain a *T. cruzi* lineage (Dm28c Cas9) expressing the protein tagged with NeonGreen. Through hydroxyurea (HU) synchronization, metacyclogenesis, and immunofluorescence techniques, it was possible to evaluate the levels of CRK1 expression in the cell cycle and metacyclogenesis, as well as its localization. The results demonstrated that the CRK1 kinase is constitutively expressed throughout the cell cycle of *T. cruzi*. On the other hand, the kinase shows reduced expression in metacyclic trypomastigote forms. Regarding its localization, preliminary results suggest a cytoplasmic localization and no nuclear localization at any time. Furthermore, CRK1 appears to have a mitochondrial localization pattern in G1/S and G2 epimastigotes. Therefore, it is considered that the dynamics of CRK1, in terms of its expression and localization, do not differ from what has been described in the literature for CDKs in other eukaryotes. **Supported by:**FAPESP_2022/08866-6 **Keywords:**CRK1;Cell cycle;Trypanosoma cruzi.

PV-31 - **Participation of the ABCA1 transporter in the cholesterol traffic in *Trypanosoma cruzi*.**

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Trypanosoma cruzi presents different developmental forms according to host where the parasite is found. The epimastigote forms are found in the digestive tract of triatomines and presents high rates of endocytic activity, absorbing molecules such as LDL (low-density lipoprotein). The uptake of LDL (cholesterol) pass through the endocytic route up to the reservosomes, where cholesterol is stored or distributed to the rest of the cell according cell demand. Part of cholesterol goes to lipid droplets (Pereira et al, 2018). The absorption, exit and distribution of cholesterol is well stablished in higher eukaryotic models. However, there is little information about cholesterol traffic in pathogenic protozoans, including T. cruzi. The ABCA1 transporter (from the ABC transporter family) participates in the reverse cholesterol transport in mammals, and it was also described in flagellar pocket membrane and reservosomes of epimastigotes (Torres et al, 2004). However, its role continues unexplored in T. cruzi. Our aim is to understand how the TcABCA1 transporter contributes to the cholesterol traffic in epimastigotes and its involvement in cholesterol exit from reservosomes to other subcellular compartments. Besides, the description of TcABCA1 in flagellar pocket opens questions about the cholesterol efflux in the parasite. After incubation of epimastigotes in medium supplemented with ABCA drug inhibitors as Cyclosporin A (0 - 20 uM) or DIDs (0- 200 uM), we observed almost 85% of reduction of LDL - TopFluorCholesterol uptake and storage in reservossomes. The parasite viability and enzymatic metabolism were assessed by MTS/PMS assay. We produced mutant parasites that expressed TcABCA1-mNeonGreen and observed the addressing to the reservosomes. Our strategy now is to produce knockout parasites TcABCA1 (-/-) in order to study the implications in proliferation and viability, LDL traffic from reservosomes to other compartments or mobilization of neutral lipids from lipid droplets. **Supported by:**FAPERJ 205.229/2022 **Keywords:**Trypanosoma cruzi;Cholesterol traffic;ABCA1 transporter.

PV-32 - **Use of the CRISPR/Cas9 to evaluate the Localization and Expression of the Cyclin 5 in epimastigotes of *Trypanosoma cruzi* throughout the Cell Cycle**

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Trypanosoma cruzi, the etiologic agent of Chagas Disease, has a complex life cycle composed of different life forms (present in invertebrate or vertebrate hosts) that vary about their replicate capacity, where infective forms do not replicate. In model eukaryotes, the replication control occurs throughout the cell cycle and is regulated by cyclin-dependent kinases (CDKs) and cyclins. In *T. cruzi*, cyclin 5 stands out, a kinase-activating protein that interacts with CRKs (homologous proteins of CDKs) 1 and 3. In addition, it presents modulations in its phosphorylation sites during the change from the epimastigotes to the metacyclic trypomastigotes forms, a process known as metacyclogenesis. Given this, cyclin 5 can be a promising target in understanding the proliferation and differentiation processes of the parasite. Thus, this work aims to characterize the cyclin 5, by generating a cyclin5-tag lineage and analysing its expression profile and location throughout the cell cycle of *T. cruzi*. For this, the CRISPR/Cas9 reverse genetics technique was used in epimastigotes to add tags Myc and Neon Green in the 5' portion of this gene of interest; and different assays were performed to validate the recombinant lineage and the protein expression and localization profile. The results showed that it was possible to generate and validate the CRISPR/Cas9 modification in the N-terminal region of the cyclin 5 protein, giving rise to the blasticidin-resistant strain cyclin5_Myc_NG. Cyclin 5 showed a possible differential expression, with higher expression in the S phase, and cytoplasmic localization, with evidence of clusters in the region around the nucleus and kinetoplast. However, further analyzes of cluster patterning and expression are being performed to validate our previous data. Obtaining the lineage allowed us to carry out further studies of localization and expression in other life forms of the parasite, in addition to the epimastigote. **Supported by:**FAPESP/ 2022/02243-7 **Keywords:**Cyclin 5;Cell Cycle ;Trypanosoma cruzi.

PV-33 - **Role of NADPH-oxidase Inhibitors in *Leishmania amazonensis* LFR1 Activities**

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Leishmania amazonensis occurs in several parts of Brazil and is the causative agente of cutaneous leishmaniasis. The parasites are transmitted by sandflies insect vectors to vertebrate hosts in the flagellated form called metacyclic promastigote. In the vertebrate host, promastigotes differentiate into aflagellated intracellular amastigotes and begin to multiply within macrophages. Recently, our group showed that heme, an essential nutrient that *Leishmania* needs to acquire from the host, activates a ferric reductase enzyme (LFR1) that has also a NADPH-oxidase (NOX) activity with high production of hydrogen peroxide (H_2O_2), and crucial importance to the differentiation of the parasite to amastigote forms. NOX enzymes are involved in several pathologies such as cancer, heart disease and neurodegenerative diseases. Therefore, there is an intense search for drugs that inhibit the activity of these molecules. Some of these inhibitors are commercially available, such as GKT137831 (Setanaxib), and GKT136901. In this context, the objective of this study is to investigate the effect of these NOX inhibitors on both LFR1 activities of *L. amazonensis* and to verify the effect of these drugs on parasites proliferation and differentiation. Preliminary results using MTT cytotoxicity test, show that the tested compounds were not able to cause cytotoxity on *L. amazonensis* after 24 hours of treatment. However, both were able to inhibit LFR1-NOX activity in a dose-dependent manner. It is possible that inhibition of LFR1 by GKT137831 and GKT136901 is involved in the differentiation of the parasite to the amastigote forms, rather than in the cytotoxicity of the promastigote forms. We are still evaluating toxic effects for longer periods of time. Also, we do not rule out the possible toxicity to amastigote forms. These and other investigations are on going in our laboratory. **Supported by:**FAPERJ Processo 211.283/2019 **Keywords:**Setanaxib;GKT136901;Ferric-reductase.

PV-34 - **The route to go: Identifying key amino acid metabolic steps as potential drug targets for Chagas disease treatment using bar-seq CRISPR-Cas9**

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Trypanosoma cruzi, the causative agent of Chagas disease, undergoes a complex digenetic life cycle, which entails environmental changes that result in substantial variations in temperature, osmolarity, and nutrient availability. Within this context, amino acids and their metabolism play crucial roles in cell differentiation, maintenance of cell volume, and response to diverse forms of stress. This study aims to identify the key steps within the amino acid metabolic network that are essential for *T. cruzi* to complete its life cycle. To achieve this goal, we are employing the bar-seq CRISPR-Cas9 genome editing strategy to generate mutant knockout cell lines for 40 enzymes that are putatively involved in amino acid metabolism. Each cell line is assigned a unique bar-code sequence, and they will be pooled together to facilitate parallel phenotyping during cell differentiation and infection, both *in vitro* and *in vivo*, employing next-generation sequencing. The relative fitness throughout the various stages of the life cycle will be assessed by quantifying barcode abundance, enabling the identification of enzymes that are vital for parasite development and infectivity. Thus far, we have successfully generated 24 mutant cell lines, comprising 12 hemi-knockouts and 12 total knockouts. Interestingly, the enzymes for which only partial knockout of the coding sequence was attainable are associated with Asn, Asp, Ser, and Thr metabolism, while complete knockout was achieved for enzymes involved in Glu, Ala, Met, Trp, and Gly metabolism. This strategy will provide insights into the metabolic pathways that *T. cruzi* has evolved to adapt to diverse environments and will also yield novel targets for future drug design. **Supported by:**FAPESP Proc n.o. 2021/12938-0, Welcome Trust Proc n.o. 222986/Z/21/Z **Keywords:**Trypanosoma cruzi;CRISPR-Cas9;Amino acids.

PV-35 - **Synthetic Biology in the Study of Protein Acetylation in Protozoan Parasites**

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Protein acetylation is present in histones and non-histones proteins and several works have been demonstrating the impact of this modification in several biological processes. Our group demonstrated the protein acetylated profile of *Trypanosoma cruzi* and *Trypanosoma brucei* and found several glycolytic and antioxidant enzymes acetylated in both parasites. Also, we validated the impact of this modification in the enzymatic activity of aldose, a glycolytic protein, and superoxide dismutase A (SODA), a key protein in the oxidative stress response. To gain insight into the role of protein acetylation in trypanosomatids we decided to establish a synthetic biology system based on the orthogonal pair pyrrolysyl-tRNA synthetase and the tRNApyl, derived from the *Methanosarcina barkeri* archaea specie, which allows the insertion of an acetylated lysine into a specific position of the protein for further biochemical analyses. To validate this system, we choose the *T. brucei* aldolase and SODA, and *T. cruzi* SODA, which was used to insert the amber codon at K157 for aldolase, and K66, K74, K97, and K101 for SODA, and cloned into pET28a vector. All the lysine sites were previously detected acetylated in our analyses. The orthogonal pair (pyrrolysyl-tRNA synthetase and the tRNApyl) together with the specific pET28a constructs were transformed in BL21 *E. coli*, and we successfully expressed the native and acetylated aldolase proteins. The acetylation of aldolase was confirmed using dot blot assays with the specific anti-acetyl-lysine antibody. In parallel, we also tested the expression of all versions of SODA acetylated at K66, K74, K97, and K101 sites. Experiments are in progress to test the enzymatic activity of acetylated aldolase and to purify the SODA proteins. In conclusion, we successfully established the orthogonal pair tool for the expression of acetylated proteins in bacteria, opening the opportunity to better understand the function of this modification in trypanosomatids **Supported by:**Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) – Processo 424729/2018-0 **Keywords:**Protein Acetylation;Synthetic Biology;Orthogonal pair.

PV-36 - **Studies of mitochondrial carriers in *L. major***

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Mitochondria are central organelles involved in many cellular processes. The inner mitochondrial membrane is impermeable to most solutes, which can only reach the matrix through the transport mediated by specific carriers. Mitochondrial carries display six transmembrane helixes with specific domains. *Leishmania* genus includes etiological agents of different forms of leishmaniasis. Most of mitochondrial carriers display low similarity to human counterparts (around 40%), representing targets for future drug design. Then we aimed to investigate if *L. major* displayed mitochondrial carriers, through bioinformatic and metabolic assessment of mitochondrial carriers in *L. major*.
Based on *Sacharomyces cerevisiae* sequences and using BLASTp algorithm we search for *L. major* sequences limiting Tritryp score to 50. From these sequences that were found for *L. major*, phylogenetic trees of maximum parsimony were constructed using Mega X program with yeast and human carriers. Based on ortology informations a table was constructed listing potential *L. major* mitochondrial carriers. A total of 37 candidates were found, expanding the previously reported inventory of mitochondrial carriers in *Leishmania*. Using ScanProsite tool conserved domains was investigated. Using online tools: Target P, Predotar and MULocDeep mitochondrial addressing was inspected. Additionally, most of the carriers were found in the proteome (TriTrypDB database), providing protein level evidence. Finally, respirometry analyses performed on intact parasites revealed that N-ethylmaleimide, a classical and non-specific inhibitor of mitochondrial phosphate carrier, significantly reduced oxygen consumption. This strongly indicates the existence of a functional mitochondrial phosphate carrier in *L. major* parasites. Based in all of these evidences we observed that *L. major* present putative mitochondrial carriers, that must be further investigated. **Supported by:**POM/FIOCRUZ e PAEF/FIOCRUZ (Lab. de Pesquisa em Leishmanioses, IOC, FIOCRUZ, Rio de Janeiro, RJ, Brazil), CNPq (308629/2021-3), FAPERJ (E-26/210409/2019).
Keywords:LEISHMANIA;PARASITIC BIOCHEMISTRY;MITOCHONDRIAL CARRIERS.

PV-37 - **Single knockout of bioenergetic enzymes leads to metabolic changes in*Trypanossoma cruzi* epimastigotes cells.**

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Trypanosoma cruzi, the pathogen responsible for Chagas disease, is transmitted by triatomine insects during their blood-feeding. Heme, a byproduct of blood digestion, plays a crucial role as a physiological oxidant molecule. It not only stimulates the proliferation of epimastigotes (a proliferative stage of the parasite) but also regulates the expression of genes associated with energy metabolism. Specifically, heme has been observed to upregulate genes involved in glycolysis and aerobic fermentation processes. Given the significance of these metabolic pathways in meeting the energy demands during this proliferative stage, this study aims to investigate the impact of genetic manipulation using the CRISPR/Cas9 system on genes related to energy metabolism in *T. cruzi* epimastigote. The gene knockout was validated by PCR and showed that only parasites lines single knockouts were obtained: glycosomal malate dehydrogenase (MDHg), glycosomal hexokinase (HKg) and cytoplasmic malic enzyme (MEc), suggesting that these genes can be essential for *T. cruzi* epimastigotes. Then, proliferation of these single knockout mutants was evaluated to determine whether it had an impact compared with both the control group, where the genes were not deleted, and the wild type cells. There was a reduction of approximately 65.7% in proliferation after seven days for the mutant of the MDHg enzyme. When conducting a proliferation curve, it was observed that the mutant showed no growth until day three, and between days four and five, it proliferated but at a rate 80% lower than the control. On the other hand, there was no difference in proliferation after seven days for the HKg mutant. Proliferation was also evaluated in the presence of Mitotempo, a reagent that removes mitochondrial ROS, and 2-deoxyglucose (2-DG), a competitive inhibitor of hexokinase. We are studying the viability and metabolism of these mutants, predicting their ability to interact with their vector and transmit the disease. **Supported by:**FAPERJ, CAPES, CNPq and INCT-EM.
Keywords: Trypanosoma cruzi;heme;energy metabolism.

PV-38 - **Structural, phylogenetic and functional analysis of E3 ubiquitin-ligase CRLs (Cullin RING-ligases) genes of Leishmania infantum**

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The ubiquitin proteasome system (UPS) is responsible for intracellular proteolysis in eukaryotes. E3 ligases play a key role in the ubiquitination process, targeting proteins for degradation by proteasome or processing by deubiquitinating enzymes. Cullin-RING ligases (CRL) are the largest and most studied class of E3 ligases in mammals. They are composed by SKP1, Cullin 1, RBX1 and an F-box protein that interacts with SKP1 through the F-box domain and recruits substrates. In parasitic protozoans with host alternation in their life cycles, intracellular proteolysis is essential for parasitism. Little is known about UPS in many parasites, including trypanosomatids of the genus Leishmania, responsible for causing leishmaniasis. Previous results from our group demonstrated that the CRL complex exists in L. infantum and the interactions between its components were validated. Here we performed in silico phylogenetic and structural analysis of L. infantum genes orthologs to the human SKP1, CUL1 and RBX1. We observed that L. infantum proteins are structurally similar to the human proteins with conserved interaction motifs among the CRLs proteins compared to their orthologs in H. sapiens. Phylogenetic analysis of orthologous proteins from 12 trypanosomatids showed that L. infantum proteins are more related to L. donovani BPK282A1 proteins, as expected. In parallel, SKP1 and Cullin1 interactome mass spectrometry of L. infantum protein extracts revealed a nucleotide hydrolase associated to SKP1 protein. We successfully cloned it in mammalian cell expression plasmids and confirmed its expression in HEK293T transfected cells. Furthermore, co-immunoprecipitation in HEK293T cells together with SKP1 will be performed. Also, a strain of L. infantum expressing myc-SKP1 and HA-hydrolase will be generated by CRISPR/Cas9 in order to demonstrate their interaction in promastigotes. Thus, these results contributes to functional characterization of CRLs in L. infantum. **Supported by:**2022/16270-6
Keywords: Cullin-RING ligase;Bioinformatics;Leishmania inf.

PV-39 - **Construction of a recyclable and compact expression vector for *Trypanosoma cruzi***

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To overexpress exogenous genes in T. cruzi some expression vectors are available such as pTEX, pRIBOTEX, pTREX, pTcINDEX, pTcGW, and pROCK. Most of the expression vectors use neomycin resistance gene, followed by hygromycin resistance, and few reports use Blasticidin or puromycin resistance genes as selectable markers. Additionally, the empty expression vectors have 7-10 kb in size, which limits the size of the transgenes that can be cloned into them. This limited number of selectable markers used and the size of the vectors prompted us to develop a new series of vectors. Aiming to construct the vectors easily, we have adapted the Biobrick strategy based on T. cruzi genome features creating the TcBIOBRICK. In the Biobrick approach, each plasmid segment is called a brick, which can be joined by using only 4 restriction enzymes including compatible end enzymes. Several segments can be assembled using the same enzyme set. Using the TcBIOBRICK approach, we build pTcMini-NG, a 5.5 kb vector for stable expression including ribosomal promoter, the mNeon Green as reporter cassette, and NeoR gene flanked by loxP sites. pTcMini-NG was stably transfected into epimastigotes, and the NG-positive parasites population was reverted to G418-sensitivity by CRE editing, i. e. by electroporation with recombinant CRE recombinase. **Supported by:**Fundação Araucária **Keywords:**Biobrick;pTcMini;plasmid.

PV-40 - **Exploring *Trypanosoma cruzi* sirtuins as therapeutic targets in Chagas disease**

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Chagas disease, also known as American trypanosomiasis, is a parasitic disease caused by the protozoan *Trypanosoma cruzi*, and endemic in Latin America, affecting more than 7 million people. During its life cycle, *T. cruzi* transits between an invertebrate and a vertebrate host and has been exposed to environmental changes that require its adaptation to survive. Acetylation is a post-translational modification of proteins present in the *T. cruzi* parasite and is regulated by two classes of enzymes: lysine acetyltransferases, which adds the acetyl group, and lysine deacetylases that removes the acetyl group from lysine residues. Sirtuins are NAD+-dependent lysine deacetylases involved in several cellular processes and have been also explored as drug targets in several diseases. Previous work from our group characterized *T. cruzi* sirtuins (TcSir2RP1 and TcSir2RP3) and demonstrated the potential to explore these enzymes as potential drug targets in this parasite. To advance in the validation of *T. cruzi* sirtuins as drug targets we used the TcSir2rp1 and rp3 purified heterologous proteins to test several biochemical parameters important for deacetylation activity, including protein concentration, reaction time, temperature, pH, substrate and NAD+ concentration. We found that in general, both enzymes had similar biochemical parameters, except for Km, Vmax and Kcat, while TcSir2rp1 has a Km=42,46 µM, Vmax=0,0170 µM.s and Kcat=0,0282 s^{-1}; TcSir2rp3 has a Km=74,11 µM, Vmax=0,0169 µM.s and Kcat=0,01 s^{-1}. Using the best deacetylation conditions we tested the inhibitory capability of a library of 23 SIRT inhibitors against TcSir2rp11 and rp3. These inhibitors were designed based on the chemical structure of previous SIRTi tested in our lab and we found that 3 and 5 compounds presented >35% of inhibitory activity, against TcSir2rp1 and rp3, respectively. Experiments are in progress to determine de IC50 of each compound and to test their effect on *T. cruzi in vitro* infection. **Supported by:**CAPES - 88887.64746/2021-00
Keywords:T; cruzi ;Sirtuins;Chagas disease.

PV-41 - **Cell division-associated targets are modulated in kharon1-deficient *Leishmania infantum***

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The disruption of *kharon1* gene in *Leishmania* spp. leads to live-attenuated amastigotes, impairing cell cycle progression due to cytokinesis defect, making the parasites unable to maintain the infection, which can be used to better understand the molecular basis of cell division. Here we select *L. infantum kharon1* null mutants (Δ*Likh1*) by CRISPR/Cas9 and evaluate by RT-qPCR the modulation of transcripts that encode for 11 cell division/cytoskeleton-associated proteins: Centrin4, Sfi-1, SAS-6, KKT, KMP-11, AIRK, KHAP1 and KHAP2, KPP1, TFK1 and NUP92. In Δ*Likh1*, Sfi1, KHAP2, TFK1, and KPP1 were upregulated in promastigotes, while amastigote forms presented increased levels of mRNA that encodes to KHAP1 and KHAP2. In order to globally evaluate the transcript modulation, we performed cDNA sequencing of Δ*Likh1*. This resulted in the identification of 10 downregulated and 71 upregulated transcripts. Among them, we selected 5 upregulated targets that were associated with cytoskeleton and cell division process (SET/SET7, PK53, COP, AP2 and CYC6). They mRNA level was validated by RT-qPCR, whose upregulation was confirmed in promastigote forms, Furthermore, the PK53 encoding mRNA was upregulated in both, promastigote and intracellular amastigote forms. It may indicate compensatory actions, suggesting that during cell division kharon1 plays a role dependent on these factors which are probably orchestrating cell division dynamics in *L. infantum*. Whether they act as a protein complex or regulating (direct or indirectly) cell division is yet to be described. Knowing that KHAP1 and KHAP2 were previously described as kharon-associated proteins 1 and 2 respectively, the subcellular localization of the proteins in promastigotes and amastigotes was performed, indicating corresponding locations of Kharon1. The basic findings here can be applied to better understand the mechanisms of chromosomal segregation and these proteins can be studied as drug targets for tackling leishmaniasis. **Supported by:**Fapemig, CAPES, CNPq
Keywords: Leishmania infantum;kharon1;cell division.

PV-42 - **Using *Trypanosoma brucei* to study *Trypanosoma cruzi* metabolism: the disturbance caused on *T. brucei* Pro consumption by expressing the *T. cruzi* His pathway insertion**

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The life cycle of *Trypanosoma cruzi* involves an invertebrate and a vertebrate host to completion, and this parasite is highly adapted to different environments. For example, when glucose is limited in the insect's gut, *T. cruzi* can consume amino acids producing ammonia. Histidine (His) is present in high concentrations in the insect's gut and the parasite can use it to generate ATP through its conversion to glutamate (Glu), a metabolite that can be produced by other sources, like Proline (Pro). This pathway consists of four enzymatic steps and coding sequences for the four enzymes are present in *T. cruzi*'s genome but not in *Trypanosoma brucei.* Aiming to investigate its biological role, we generated *T. brucei* procyclic cell lines able to express active versions of the first two enzymes of *T. cruzi*'s His degradation pathway. These lineages did not show differences in proliferation or ATP content in the presence of His as the solely metabolite of source compared with parasites in the absence of any metabolite (control). However, when Pro is used as the only metabolite, ATP levels were diminished when compared to wild-type controls. Additionally, O_2 consumption triggered by Pro diminished by 16% when compared to the control, while His did not affect O_2 consumption. The inner mitochondrial membrane potential did not show differences between wild-type and obtained lineages. We also obtained *T. brucei* cell lineages expressing the complete His degradation pathway which are under phenotypic analysis. Our findings using *T. brucei* as a scaffold for analyzing *T. cruzi* metabolic pathways are providing valuable insights into the functional implications of the *T. cruzi* histidine-glutamate pathway and its potential impact on parasite adaptation. **Supported by:**FAPESP - 2020/16569-6 **Keywords:**Trypanosoma cruzi;Histidine;Proline.

PV-43 - **Metabolic model curation for *Trypanosoma cruzi* and *Trypanosoma brucei***

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Genome-Scale Metabolic Models (GEMs) are computational tools that employ systems biology concepts to analyze the metabolic capabilities of organisms. These models combine genomic, biochemical, and physiological data to forecast metabolic behavior. Our objective was to create models for *Trypanosoma brucei* and *Trypanosoma cruzi* as tools to provide a descriptive and predictive outcome to enhance our comprehension of the metabolism of these important parasites. GEMs help to examine how metabolic networks are established around their constituent reactions and metabolites, facilitating the understanding of the subtle relations between reactions directly or indirectly related. Our models were made using COBRAToolBox and are now being manually curated. Curation involves in-depth research on the parasites' metabolism to identify genes and their related reactions, the main goal is to work on the amino acid metabolism for both models and to understand metabolic differences between *T. cruzi* and *T. brucei*. Our *T. cruzi* model has 559 reactions, 628 metabolites and our *T. brucei* model has 302 reactions and 497 metabolites. When evaluated for consistency, they showed values of 60% and 80%, respectively. Validation of these models involves simulating metabolic scenarios to generate predictions that can be experimentally confirmed. We will compare ATP productions and metabolite secretion from *in vitro* data to the model predictions and use this data to make the models more robust. To validate the *T. brucei* model we are generating *T. brucei* cell lines *in vitro* that express the histidine-glutamate pathway, which coding sequences for enzymes are present only in *T. cruzi's* genome. By comparing experimental results with model predictions, we aim to assess the impact of introducing a different pathway on metabolic flow. Analyzing the differences between these parasites will provide insights into their metabolism and adaptations to diverse environments. **Supported by:**FAPESP - 2020/16569-6
Keywords: Genome-Scale Metabolic Models;Trypanosoma;Curation.

PV-44 - **CHARACTERISATION OF A GENE ENCODING A TRYPSIN-TYPE SERINE PEPTIDASE IN *Trypanosoma cruzi* THAT PLAYS A ROLE IN METACYCLOGENESIS**

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In *Trypanosoma cruzi*, the differentiation of epimastigotes to infective metacyclic trypomastigotes occurs in the digestive system of the Triatomine bug upon nutritional stress. Parasite differentiation involves extensive remodelling both at the morphological and molecular levels, and proteolysis is thought to enable the degradation of epimastigote-specific proteins during this process. We have reported the identification of genes encoding an inhibitor of S1A-family trypsin-fold serine peptidases in the Tri-tryps, which contribute to the infectivity of *T. brucei*, *Leishmania sp*. and *T. cruzi*. However, no genes for putative S1A peptidases were identified in their genomes. Here, we describe the identification of a gene encoding a putative trypsin-type enzyme in *T. cruzi* (*TPS*), which is similar to trypsin-2 type serine peptidases present in bacteria. The alpha-fold model of the coding sequence predicted a protein with a N-terminal trypsin-type domain, followed by an alpha-helix rich region. To investigate its biological function, we generated *T. cruzi* Dm28 null-mutants (∆*tps*) and transgenic lines where the endogenous gene was fused to the mNeonGreen_Myc reporter using CRISPR/SpCas9. Western blotting of parasite lysates using antibodies to Myc showed that *TPS:mNeonGreen_MyC* is expressed in all life-stages. Fluorescence microscopy revealed that TPS is localised at the flagellum. ∆*tps* epimastigotes grow normally *in vitro*, but start to differentiate to metacyclic trypomastigotes prematurely (mid-log phase), while no significant alterations in the cell cycle were observed. Furthermore, at stationary phase ∆*tps* present higher percentages of metacyclic trypomastigotes as compared to the Dm28SpCas9 parental line. Tissue culture trypomastigotes ∆*tps* infect mammalian cell in vitro, but the intracellular burdens of amastigotes at 48hrs are slightly lower than that od the parental line. Collectively, our data suggests that TPS modulates parasite differentiation.
Supported by:FAPERJ e CNPq **Keywords:**Cruzi;Serine Peptidase ;Differentiation.

PV-45 - **MOLECULAR APPROACHES FOR CHARACTERIZING PROCESSING BODIES IN *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi (Tc) is exposed to multiple environmental changes throughout its life cycle, demanding precise regulation of gene expression for the establishment of infection. In trypanosomatids, this regulation occurs predominantly at the post-transcriptional level, with mechanisms that stabilize messenger RNA (mRNA) playing a crucial role. Among these mechanisms, one may find processing bodies (PBs), complexes of RNA-binding proteins (RBPs) associated with untranslated mRNA that play a fundamental role in the storage and accessibility of the translation machinery to mRNA, and represent one of the most relevant mechanisms for the regulation of gene expression in Tc. TcDHH1, an RBP found in PBs, is widely used as a marker for this structure. Despite the intense investigation of the structural organization of Tc using various microscopy techniques, little is known about the ultrastructure of PBs. Therefore, this study aims to characterize the ultrastructure of PBs and investigate their interactions with other parasite organelles. We employed two main approaches: 1) generation of mutant parasites using the CRISPR/Cas9 system, and 2) electron microscopy analysis and correlative microscopy. Through PCR reactions, we amplified the SgRNA templates used in in vivo transcription and the donor DNA to generate labeled parasites (TcDHH1::mNG) or gene knockout parasites (TcDHH1-KO). After transfection with SgRNA templates and donor DNA for generating mutant parasites, we evaluated the fluorescence of the labeled parasites using flow cytometry and fluorescence microscopy, confirming the expression of the fluorescent protein with a similar localization reported in previous works using immunolocalization. The knockout parasites are undergoing selection. Subsequent steps include the validation of gene editing by PCR and Western blot. Electron microscopy characterization of the densities found by fluorescence microscopy is currently under development in our laboratory. **Supported by:**CNPq - 126068/2022-4
Keywords: Processing bodies;CRISPR-Cas9;Correlative microscopy.

PV-46 - **Is the Latitudinal Diversity Gradient applicable for unicellular eukaryotes? A Comparative Study of Arcellinida (Amoebozoa) in Brazilian Amazon and Temperate Mosses**

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The paradigm for microbial diversity is ‘Everything is everywhere, but the environment selects’. Moreover, the latitudinal diversity gradient (LDG) is a well established pattern of global species distribution. However, most microbial morphotypes (along with their ecology) have not been well characterized, and microbial diversity in the tropics is essentially unknown. Shelled amoebae form a paraphyletic free-living unicellular eukaryotic group that produces shells with taxonomic relevance. In this work in progress, we aim to compare the Arcellinida shell diversity of a moss sample from the pristine Amazon (Serra do Imeri) with the diversity found in mosses from the temperate regions. Alpha diversity, relative abundance and shell morphology of Arcellinida (Amoebozoa) will be analyzed for comparison. We identified taxa through the use of optical microscopy and scanning electron microscopy (SEM). Alpha diversity and relative abundance has been calculated with species curves. We compared the morphotypes found with previous taxonomic descriptions and SEM images. We found up to 3 times more Arcellinida taxons than in recent registers from mosses of temperate regions. More than 130 SEM pictures from different individuals were taken. Some shells of characteristic rare species (e.g. *Apodera vas* and *Certesella martiali*) showed phenotypical novelties. Based on our preliminary findings, we found evidence supporting the applicability of the LDG for the group in terms of species richness, however, some registers of rare morphotypes with novel phenotypes reinforces the perspective that molecular information from neotropical populations needs to be accessed to discuss if the lineages found in the tropics are the same as the registered in temperate zones.
Supported by:CNPq **Keywords:**Biogeographical comparison;Microbial diversity;Shell morphology.

PV-47 - **MAPPING THE *Trypanosoma cruzi* PROTEIN KINASE GENES UNIQUE AMONG THE TRI-TRYPS**

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Protein kinases (PK) are ubiquitous enzymes that transfer phosphate groups to proteins. In the pathogenic trypanosomatids, Trypanosoma brucei and Leishmania mexicana, kinome-wide screens identified several PK genes that are essential for parasite viability or required for infectivity in experimental models. Even though the sequence of the T. cruzi genome was made available nearly 20 years ago, the function of PKs has been underexplored in this organism. The putative function of PKs that are highly conserved among the tri-tryps could be potentially inferred in T. cruzi based on the phenotypes found in the PK-wide RNAi screen in T. brucei and the CRISPR/Cas9 genome-wide PK screen performed in L. mexicana. However, PK genes exclusive to T. cruzi have not been previously addressed. We performed a search in the available T. cruzi genomes for putative PK genes and identified approximately 41 genes that are absent from the available Leishmania genomes and 18 PK genes (PK1-18) that are absent from both Leishmania and T. brucei, and were thus considered T. cruzi unique. The 18 T. cruzi unique PKs are distributed among the STE, GMGC, and CAMK groups, and for one PK no matching group could be identified. Orthologues for those genes are present in a few other trypanosomatid species, e.g. T. conorhini, T. theileri, and T. melophagium, often sharing high sequence similarity. Structural modelling using alpha-fold predicted very high confidence for PK domains (>90% confidence) for 15 PKs while models were unavailable for 3 PKs. Seven T. cruzi unique PKs have orthologues in less than 5 Trypanosoma species. We have attempted to generate gene knockouts for those 7 genes, successfully generating null mutants for 3 and only heterozygotes for the other 4, suggesting their essentiality. Subcellular localisation was mapped by endogenous tagging using mNeonGreen_MyC as a reporter gene, revealing diverse distribution. Expression of the PKs in the parasite life stages is under investigation.
Supported by:faperj, cnpq, ukri **Keywords:**kinase;cruzi;function.

PV-48 - **Analysis of nascent transcripts in *Trypanosoma cruzi*.**

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Trypanosomatids have a genomic structure organized in polycistronic regions, and specific promoter sequences for each gene have not been described yet in *Trypanosoma cruzi*. Post-transcriptional mechanisms are the main ones responsible for regulating gene expression in these organisms. However, nascent transcription assays in specific genes indicate that some loci are transcribed at different rates. The present work aimed to understand the real impact of transcriptional regulation concerning the abundance of transcripts from different genomic regions using large-scale assays of nascent transcripts, known as *Global Run on sequencing* (GRO-seq). We first established a suitable computational pipeline to process and analyze nascent transcripts from different genomic regions of *T. cruzi*. Abundances were assessed by approaches based on coverages or on counting reads. Our data confirm that the GRO-seq data are enriched in nascent and unprocessed/degraded transcripts compared to the pool of mature RNAs detected by RNA-seq assays. It was observed that most PTUs have similar transcription rates. However, for some subsets of PTUs, significant differences were detected. PTUs and CDSs from the core genomic compartments are more abundant than those from the disruptive compartment. Furthermore, we detected a positive correlation between levels of chromatin opening and nascent expression, which is more evident when comparing genomic compartments. Taken together, our data point to a differential transcriptional regulation between genomic compartments associated with chromatin opening, suggesting an essential role for chromatin in the regulatory mechanisms of gene expression in *T. cruzi*. **Supported by:**Processo Fapesp 2018/15553-9 **Keywords:**Trypanosomatids;nascent transcription;computational pipeline.

PV-49 - **Biogeographic and temporal origin of Leishmania Ross, 1903 (Trypanosomatidae)**

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Several hypotheses have been proposed for the origin of *Leishmania*, based mainly on vector distribution and different hosts. The Neotropical hypothesis suggests the origin of *Leishmania* in the Neotropical region, and mammals would be their first vertebrate hosts. The Palearctic hypothesis places an origin in the Palearctic region and reptiles as their first vertebrate hosts. The Supercontinent hypothesis proposes an origin before the Gondwana breakup, with mammals as their first vertebrate hosts. The aim of the present study is to discuss the geographical origin of *Leishmania*, suggesting several processes that may have influenced current patterns of distribution of the genus based on hypotheses of phylogenetic relationships and methods of reconstructing ancestral distributional areas and molecular clock. Gene sequences from gGAPDH, HSP70 and V7V8 were retrieved from NCBI and TriTrypDB in a total of 54 terminal taxa. Genes were aligned on ClustalX and MAFFT7. Evolutionary models were accessed on jModelTest and Bayesian analyses were performed on MrBayes 3.2.7a. Reconstruction of ancestral areas was performed on RASP 4.2 using a Statistical Dispersal-Vicariance analysis. A dating analysis using relaxed molecular clock was performed on BEAST 2.5, with *Trypanosoma* as outgroup and data from *Paraleishmania* fossils. Bayesian analysis recovered *Leishmania* and its subgenera as monophyletic (pp=100%). S-DIVA analysis recovered as ancestral area of origin of *Leishmania* in the Neotropical region (p=35%), or Neotropical+Oriental region (p=34%) or Afrotropical+Neotropical+Oriental region (p=31%). Geographic events recovered by S-DIVA analysis corroborate the Supercontinent hypothesis. Initial molecular clock analyses show that the *Leishmania* parasites first appeared at about 80 mya, which suggests that they may have been originated before the Gondwana breakup. **Supported by:**CNPq
Keywords: Molecular Clock;Molecular Phylogeny;Biogeography.

PV-50 - **Sauroleishmania Ranque, 1973 emend. Saf'janova, 1982 (Leishmania: Trypanosomatidae): Bibliographic and phylogenetics review of the subgenus**

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Leishmania is a member of the subfamily Leishmaniinae and has three subgenera of medical and veterinary importance: *Leishmania*, *Viannia* and *Mundinia*. While those three subgenera infect mammals, *Sauroleishmania* is the only one described to infect reptiles. *Sauroleishmania* has a distribution restricted to the Old World and consists of 19 recognized species. These species are transmitted by sandflies of the genus *Sergentomyia* (Diptera: Psychodidae). The aim of the present study was to gather information in the literature about *Sauroleishmania* and to discuss the phylogenetic relationships between the species of this subgenus. Bibliographic data on *Sauroleishmania* were collected using several search tools, from its first citation to the most recent data. Sequences from HSP70 gene (619 bp) were retrieved from NCBI in a total of 45 terminal taxa (29 from *Leishmania*) and were aligned on ClustalX implemented on MEGA 10.1.8. Evolutionary model was accessed on IQ-Tree 2.2.2.6. The method of Maximum Likelihood was performed on MEGA 10.1.8 with 50 replications and GTR+I+G model. Maximum Likelihood analysis recovered *Sauroleishmania* as monophyletic, with 100% support and forming a sister group with the subgenus *Leishmania* (96%). Our study also used sequences from three undescribed lineages that cause visceral leishmaniasis in a province of China, and they were recovered in the same clade as other species of *Sauroleishmania*. Furthermore, only four species of the 19 subgenera of *Sauroleishmania* possess additional information beyond the initial description. For example, *L. (S.) henrici* has only one article from 1918 and none type material deposited in collections. The subgenus has many questions and little information about its species, both in the literature and in molecular databases. *Sauroleishmania* is composed of non-pathogenic species for mammals, which makes them an important model for molecular and evolutionary studies on the origins of *Leishmania* pathogenic species. **Keywords:**Leishmania (S;) henrici;Reptiles;Molecular Phylogeny.

PV-51 - **Disrupting Ubiquinone Biosynthesis in Malaria Parasites: a new strategy to amplify the effects of atovaquone**

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One of the most promising targets for malaria therapy development is the biosynthesis of isoprenoids. There are several metabolites that are important for *P. falciparum* and that are isoprenoids, but this study concentrates on the metabolism of only one of them: ubiquinone (or coenzyme Q; UQ). One of the most recently discovered antimalarials, atovaquone (ATO), is an inhibitor of mitochondrial complex III by competing with the reduced form of UQ, ubiquinol (UQH_2). This process shows that the availability of UQ/UQH_2 is critical for parasite respiration, which unlike in the human host, is precisely essential for pyrimidine biosynthesis. Therefore, this work aimed to evaluate the importance of the UQ biosynthesis pathway in the parasite by using inhibitors and creating an inducible knockdown line of PfCOQ2, predicted to codify a 4-hydroxybenzoate polyprenyl transferase (4-HPT), the first step of the UQ biosynthesis pathway. The reduced gene expression was found to affect the parasites' long-term viability and trigger increased susceptibility to ATO. Analogues of 4-hydroxybenzoate (4-HB) demonstrated UQ biosynthesis inhibitory action, specifically affecting the recombinant 4-HPT activity and potentiating the ATO antiplasmodial effect. Finally, it was possible to propose that alterations in the radicals linked to the C4 atom of the 4-hydroxybenzoate phenyl ring are more efficient than those linked to the carboxylic group in promoting the potentiating effects of ATO. These results open the way for the development of an antimalarial pharmaceutical formulation containing ATO that is more efficient than the one currently used. **Supported by:**FAPESP 2022/09526-4
Keywords: Malaria;atovaquona;ubiquinona.

PV-52 - **IDENTIFICATION OF A *Trypanosoma cruzi* PROTEIN KINASE GENE THAT PLAYS A ROLE IN METACYCLOGENESIS**

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Protein kinases (PKs) exert fundamental roles in cells, including signalling, replication, and differentiation. In the pathogenic trypanosomatids, Trypanosoma brucei and Leishmania mexicana, genome-wide screens revealed several essential PK genes and other PKs required for infection. In the genome of T. cruzi, we have identified eighteen putative PK genes (PK1 to PK18) that are absent from T. brucei and Leishmania, and thus were considered T. cruzi unique. PK5 was predicted to be from the STE group, and orthologues are present in T. rangeli, T. cruzi marinkellei (infects bats), T. conorhini (infects rats), T. theileri (infects bovines), and in T. melophagium (infects sheep). To investigate its function, we have generated null mutants (Δpk5) in T. cruzi Dm28 epimastigotes, as well as transgenic lines where the endogenous PK5 was fused at the C-terminus with mNeonGreen_MyC, using CRISPR/SpCas9. The expression of PK5:mNeonGreen_MyC was evaluated through Western blot, showing that it is expressed in epimastigotes but it is not expressed in tissue culture trypomastigotes. In epimastigotes, the protein has punctate distribution throughout the cell body. Δpk5 epimastigotes grew normally in vitro, but were unable to differentiate spontaneously to metacyclic trypomastigotes during stationary phase (up to day 12), in contrast with the SpCas9 parental Dm28 line. After 28 days, elongated forms were observed in Δpk5 cultures, which were resistant to lysis by human serum, suggesting that PK5 is required for timely differentiation. **Supported by:**Faperj, CNPq e UKRI
Keywords: Kinase;Cruzi;Mtetacyclogenesis.

PV-53 - **The knockout of *Leishmania braziliensis* Arginine Methyltransferase 5 alters the transcriptome of metacyclic promatigote forms and reduces amastigogenesis *in vitro***

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Leishmania braziliensis is the major causative agent of tegumentary leishmaniasis in South America. During its life cycle, the parasite alternates between several morphologies, most remarkably procyclic and metacyclic (META) promatigote forms (in the phlebotomine) and the amastigote (AMA) form (in the mammalian host). An efficient transition from META to AMA is particularly important for the establishment of the infection in the host cell. It was recently demonstrated in our lab that the knockout (KO) of the Arginine Methyltransferase 5 (*Lbr*PRMT5) in *L. braziliensis* reduces its ability to infect macrophages and to undergo amastigogenesis *in vitro*. To understand the molecular mechanisms coordinated by *Lbr*PRMT5 that are implicated in amastigogenesis, we performed a RNAseq analysis of the META forms of PRMT5-KO in comparison to its parental line (pT007), as well as to a PRMT5-addback line. PCA analysis showed that the alterations in the transcriptome of PRMT5-KO cells were largely restored in PRMT5-addback cells. Importantly, only six genes were found downregulated in PRMT5-KO META forms, while 88 were found upregulated. GO terms related to ncRNA and to macromolecule metabolism were enriched among the upregulated genes, but no enrichment was observed for the downregulated sequences. Using a CRISPR/Cas9 approach, we attempted to knockout all the downregulated genes to assess whether they would be determinant for the META-AMA transition. At this stage, one gene was excluded from the analysis for showing characteristics of transposable elements, and two genes appeared essential for *L. braziliensis* growth and could not be knocked out. The remaining three genes were knocked out: the KO of two (LBRM2903_160008200 and LBRM2903_160019400) reduced the rate of *in vitro* amastigogenesis by ~30%. The double KO of these two genes did not seem to reduce axenic amastigogenesis further. The ability of the KO cell lines to infect macrophages *in vitro* is being assessed. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo **Keywords:**Leishmania braziliensis;Arginine Methyltransferase;PRMT5.

PV-54 - **Does ATM play a role in DNA repair and genome plasticity in *Leishmania*?**

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To ensure the genome is faithfully transmitted to the next generation, DNA injuries or lesions must be resolved. Failing to repair this damage can promote chromosome breaks, genome instability, tumorigenesis, and cell death. Double Strand Breaks (DSBs) are highly deleterious lesions where both DNA strands are compromised. In eukaryotes, specialised pathways resolve DSBs as part of the wider Damage Response (DDR). Ataxia-telangiectasia Mutated (ATM), is crucial kinase in coordinating DSB repair that is recruited to and activated at DSB sites by a complex called MRN (Mre11-Rad50-Nbs1). Once ATM activates, it triggers a phosphorylation cascade stimulating the recruitment of additional repair factors and promoting accurate DSB repair. In *Leishmania*, a pathogenic protozoan that causes leishmaniasis, recent studies suggest ATM resolves oxidative stress and may play a role in parasite differentiation, but more work is needed to examine the scope of ATM's functionality, particularly during DSB repair in these parasites. We used CRISPR/Cas9 to delete ATM in *L. major* promastigotes, finding that ATM, *in vitro*, is non-essential but loss of the kinase affects parasite proliferation, particular under genotoxic stress conditions, leading to the activation of a previously undescribed damage induced G1/S phase checkpoint. Loss of ATM also coincided with the accumulation of RPA1 foci and enhanced DSB formation, supporting a role for ATM in maintaining genome stability. Given extrachromosomal amplification in these parasites is associated with genome instability, we tested whether ATM may play a role in this process. Indeed, we found that loss of ATM leads to a loss of circular DNA amplification, with future work aimed at resolving this parasite-specific function. **Supported by:**FAPESP **Keywords:**ATM kinase;Double strand breaks;genome instability.

PV-55 - **Unveiling a novel Isoprenoid Salvage Pathway in *Plasmodium falciparum*: new perspectives in fosmidomycin as an antimalarial**

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Malaria, predominantly caused by the *Plasmodium falciparum* pathogen, remains an endemic problem in tropical and subtropical regions. The emergence of drug resistance is a major problem, highlighting the need for new antimalarial compounds. Fosmidomycin (FOS) inhibits the methylerythritol 4-phosphate (MEP) pathway, crucial for isoprene unit biosynthesis. Despite its efficacy, recurrent cases of recrudescence have led to investigations into the biological causes of FOS recovery. This study found isoprenoid alcohols such as farnesol (FOH), geranylgeraniol (GGOH), phytol (POH), and unsaponifiable lipid extracts from foods can restore FOS activity in *P. falciparum*. These substances are phosphorylated, lengthened, and incorporated into proteins. Proteomic and radiolabeling studies showed that prenylated proteins can bind to several isoprenoids if externally supplied. A gene (Pfpolk) encoding a prenol kinase was identified, which when expressed in yeast, exhibited farnesol and geranylgeraniol kinase activities. Conditional knockout parasites (ΔPfpolk) were created using CRISPR-Cas9 and DiCre strategies, to investigate the biological importance of the farnesol/geranylgeraniol salvage pathway. ΔPfpolk parasites were more susceptible to MEP inhibitors and incapable of using isoprenoid alcohols for protein prenylation. The study suggests that the farnesol and geranylgeraniol salvage pathway is an additional isoprenoid source for malaria parasites. Inhibition of this pathway could enhance the effectiveness of drugs targeting isoprenoid metabolism. A compound reducing the FOS-recovering effect of geranylgeraniol was identified, making it a potential candidate for co-use with FOS in trials. Collectively, these findings deepen our understanding of the action mechanisms of antimalarials targeting the apicoplast and shed light on a novel post-translational modification of proteins in *P. falciparum*, providing valuable insights for the development of more effective antimalarial drugs. **Supported by:**FAPESP 2018/02924-9 and 2020/14897-6 **Keywords:**Malaria ;fosmidomycin;isoprenoid.

PV-56 - **U18666A: A tool for understanding intracellular cholesterol trafficking on *Leishmania amazonensis***

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Leishmaniases are part of the neglected tropical diseases caused by different species of protozoa of the genus *Leishmania*. Sterols are structural lipids and are present in the membranes of most eukaryotic cells. Unlike animals, which synthesize cholesterol, *Leishmania* spp. synthesize ergosterol and other ergostane-based sterols. In addition, this parasite can obtain exogenous cholesterol derived from the culture medium or the host cell. Therefore, studying cholesterol uptake by *Leishmania* spp. is fundamental for understanding the role of this sterol in the disease. To develop this study, an amphipathic cationic amine known as U18666A [3β-(2-diethylaminoethoxy)androst-5-en-17-one] was selected. This chemical compound can alter intracellular membrane protein traffic by impairing intracellular biosynthesis and transport of LDL-derived cholesterol. Because of this, it is widely used to address questions about the control of cholesterol biosynthesis, intracellular cholesterol traffic, and traffic of other molecules through endosomal and lysosomal compartments, and in understanding the pathophysiology of various diseases, including epilepsy, cataract, Niemann-Pick type C disease, atherosclerosis, Alzheimer's disease, prion infections, and others. In this work, the efficacy in *Leishmania amazonensis* promastigotes was evaluated. The IC50 in promastigotes of *L. amazonensis* were 0.63 ± 1.0. Cytotoxicity assays of the compound U18666A on macrophage cultures revealed that the compound alone is not toxic to uninfected peritoneal macrophages at the concentrations tested. Trials on amastigotes have shown promising results. This work indicates that U18666A has antileishmanial activity and further studies will evaluate its activity on the sterol composition of the parasite and the effect in combination with azoles. **Supported by:**Faperj E26-201.158/2022 e Faperj E26-210.157/2018 **Keywords:**Leishmania amazonensis ;U18666A;Sterols.

PV-57 - **Insights on The Role of the PAF and LPC Receptor in Cell Biology and Mitochondrial Physiology of Trypanosoma cruzi**

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Phospholipids, such as platelet-activating factor (PAF) and lysophosphatidylcholine (LPC), mediate proliferation, cell differentiation, and infectivity in diverse eukaryotes, including trypanosomatids. Our group identified a gene encoding a putative PAF and LPC receptor in *Trypanosoma cruzi* (TcPAQR) and produced single knockout (SKO) and total knockout (KO) mutants for this gene. Previous ultrastructural analysis revealed alterations in the morphology and mitochondrial ultrastructure of KO parasites when compared to WT cells. Thus, the objective of the present study was to evaluate the importance of this receptor in cell biology and mitochondrial physiology in *T. cruzi*. Comparing the growth curves over 7 days by direct counting, the SKO and KO parasites were found to have a significantly lower proliferative capacity from the 5th day onward. The cell viability of the three phenotypes (WT, SKO, KO) was compared, and the results indicate that all protozoa were viable throughout the experiments. The apoptosis rate of these parasites was also evaluated by the TUNEL method and flow cytometry. The results showed a higher rate of apoptosis for KO compared to WT and SKO parasites. To assess the oxygen consumption of the three phenotypes (WT, SKO, KO), an analysis was performed by respirometry, using modulators of mitochondrial processes, showing that there were no significant differences between the three phenotypes. This indicates that, despite possible damage to mitochondria, the respiratory chain is likely to be functional. However, KO mutants showed a reduction in mitochondrial membrane potential and a high ROS content, which suggests a mechanism yet to be studied to sustain oxygen consumption at normal levels and thus remain alive. This work highlights the importance of the receptor for PAF and LPC in the basic survival mechanisms of *T. cruzi*, which can be explored as a potential target for experimental chemotherapy against Chagas disease. **Supported by:**CNPq, CAPES, FAPERJ, INCTEM **Keywords:**Mitochondria;PAF and LPC receptor;Mutants.

PV-58 - **In silico Characterization of TcPAQR: A Putative Receptor for PAF and LPC in *Trypanosoma cruzi***

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TcPAQR is a homologous protein to members of the progestin and adiponectin family of receptors (PAQRs) in humans. TcPAQR is a transmembrane protein and is considered a putative receptor for the lipid mediators platelet-activating factor (PAF) and lysophosphatidylcholine (LPC), which play a role in *Trypanosoma cruzi* cell differentiation and the infection of peritoneal mouse macrophages. In vitro assays have shown that knockout parasites lacking TcPAQR4 were unable to respond to the stimuli of PAF and LPC in cell differentiation. The main goal of this project was to discover possible drugs to render the infection nonviable in humans. TcPAQR was modeled using AlphaFold, an Artificial Intelligence (AI) program developed by Google DeepMind, known for its accurate protein molecular modeling, especially for proteins with low similarity to homologous structures (<30%). Additionally, modeling was performed using RaptorX, I-Tasser, Modeller, and Phyre2, which are programs based on the structure of known homologous proteins. Modeller required a pdb file for modeling, and the 6KS1 sequence was utilized. For AlphaFold, ColabFold was employed, applying 6 recycling steps, the Amber ForceField, and the pdb70 database. All other settings were left as default. All models were compared using RMSD analysis in PyMOL, and a structure validation analysis was conducted using Saves (UCLA-DOE), which employed VERIFY 3D, ERRAT, PROVE, WHATCHECK, and PROCHECK. Based on the analysis, the AlphaFold model was deemed the closest approximation to reality. Molecular docking of this receptor using Vina will be performed in the near future. **Supported by:**CNPq, CAPES, FAPERJ, INCTEM **Keywords:**PAF and LPC receptor;Molecular modeling;Artificial intelligence.

PV-59 - **One Health Approach to Tackle Cryptosporidiosis and Giardiasis in the Pampa Region of Argentina**

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Cryptosporidiosis and giardiasis are emerging enteritis diseases with zoonotic risks globally. *Giardia and Cryptosporidium primarily impact young individuals, leading to malnutrition and significant economic losses in livestock.* In this work, we aimed to assess *Cryptosporidium* and *Giardia* presence and to identify circulating subgenotypes and risk-associated factors in Córdoba, Argentina. Our study evaluated the copro-parasitological status of 150 asymptomatic children under 15. We collected 374 soil samples, and 493 dog fecal samples, and processed fecal samples from 140 calves under 60 days of age from two dairy farms in the study area. We also assessed nutrition, demographics, and anthropometric data using questionnaires and census information. Dog variables included characteristics, health status, environment, anthelmintic use, veterinary management, and owners' commitment. Data were analyzed using multi-level models, AIC comparison, and generalized linear mixed-effects models in R. Geographic information systems helped create predictive maps for parasitic exposure, malnutrition, animal infections, and combined conditions. In children, five genera of parasites were detected, with *G. duodenalis* being the most common pathogen. Individual and household factors influenced the presence of *Cryptosporidium* and *Giardia*, while neighborhood-level environmental factors also played a role in co-infection. Calves predominantly showed *G. duodenalis* genotype E, and several subgenotypes of *C. parvum* were identified, including a novel subgenotype. The most frequent subgenotype in calves with diarrhea was IIaA20G1R1, followed by IIaA18G1R1. These subgenotypes were also found in humans, indicating a potential for zoonotic transmission. The study's findings are significant for understanding the zoonotic potential of gastrointestinal protozoan parasites and their impact on public health in the Córdoba province, highlighting the importance of One Health approaches. **Supported by:**Agencia Nacional para la Promoción de la Ciencia y Tecnología, Argentina, grant number PICT2018-713 and PICT-2021-CAT-II-00073.
Keywords:0NE HELTH;parasitic infections;zoonosis.

PV-60 - **Investigating Growth Patterns and Species Interactions of Trypanosomatid Parasites in a Cultured Environment**

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In this study, individual growth curves were generated for five different parasites, namely *Strigomonas oncopelti*, *Crithidia fasciculata, Leishmania amazonensis*, *Phytomonas serpens*, and *Trypanosoma cruzi*. The parasites were cultured in liver infusion tryptose (LIT) medium supplemented with 0.002% hemin and 10% heat-inactivated fetal calf serum at 28°C, pH 7.2. An inoculum of $5x10^5$/ml in 5 ml was used. Subsequently, all five parasites were combined and added to the same culture medium ($5x10^5$/ml in 5 ml). The parasite pool was harvested daily, and the culture medium was replaced with fresh medium. Each species of trypanosomatids was counted daily for a period of 14 days using a Neubauer chamber. The resulting percentages of each parasite were plotted. To further investigate this phenomenon, a similar experiment was conducted using only three species (*S. oncopelti*, *P. serpens*, and *T. cruzi*), and the same pattern emerged. *T. cruzi* disappeared from the culture around day 6-7, while *P. serpens* was found in very low numbers from day 11 onwards. *S. oncopelti*, on the other hand, persisted in high numbers until the 14^{th} day of culture. Each experiment was replicated five times in triplicate. Currently, ongoing experiments are being conducted to shed light on the mechanisms underlying this phenomenon. **Supported by:**CNPq, CAPES, FAPERJ, INCTEM **Keywords:**Strigomonas oncopelti;Phytomonas serpens;Trypanosoma cruzi.

PV-61 - **Biochemical characterization of cytokinin biosynthesis in *Plasmodiu ciparum***

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Malaria is a parasitic infectious disease caused by P th problem and a risk to diverse populations. Pre ase poses a threat to 40% of the world&#39 primarily observed in isolates of *Plasmodiu* drugs. In this regard, the biosynthesis of iso gained prominence as a potential target for th s disease. This is due to the fact that the co survival of any cell, and the biosynthetic p iosynthesis pathway in humans. Isopreno nesis regulation in plants. Therefore, we aim in the intraerythrocytic forms of *Plasmodium* egulating the parasite's cell cycle. Our results nesis in *Plasmodium falciparum*, and we observed the e duced cytokinin biosynthesis and viability in P. falciparum, as we ization. The compound forchlorfenuron decreased parasitemia and velopmental stages of the P. falciparum cycle. Understanding the bioch in cytokinin biosynthesis will contribute to a better understanding of cytokinin functio *sp.*, and guide the development of new therapies for the treatment of malaria with *Plasmodium falciparum*. **Supported by:**CAPES processo: 88887.473720/2020-00 (PROE , **Keywords:**Malaria;phytohormones;cytokinins.

PV-62 - **Prospection of C24-methyltransferase (ERG6) inhibitors for the treatment of leishmaniasis**

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In searching for the rational development of new drugs, choosing a selective target in the parasite is essential. The divergent step of the parasite lipid biosynthesis pathway is the transfer of one methyl to carbon 24 of sterols with a cholestane structure, forming a branch in C24 not existing in mammalian sterols catalyzed by the C24-sterol methyltransferase enzyme (ERG6). This work aims to evaluate azasterols with different modifications in their structure as inhibitors of the enzyme ERG6 of *Leishmania* spp. For this purpose, we used strains of *Leishmania infantum* and *Leishmania amazonensis* that overexpress ERG6 to perform a drug screening. The azasterols evaluated in this work were synthesized by our collaborators. Finasteride and dutasteride were acquired from Sigma-Aldrich and also had their leishmanicidal activity evaluated for belonging to the class of azasteroids. Confirmation of increased in gene transcripts in both *Leishmania* strains was confirmed by quantitative PCR. In the transfected promastigotes of *L. amazonensis* (Laerg6^{high}), it was possible to observe an increase of the relative expression of mRNA of ERG6 of approximately 17 times compared to the wild strain, while in *L. infantum* strain (Lierg6^{high}) we observed an increase of approximately 45 times. Then, promastigotes Lierg6^{high}, Laerg6^{high}, *L. amazonensis* WT and *L. infantum* WT were incubated with test molecules at different concentrations and with dutasteride and finasteride for 72 hours. Afterward, the growth of parasites was evaluated with resazurin. All azasterol derivatives of the ND series showed leishmanicidal activity against promastigotes of *Leishmania* spp. Additional tests will be performed with the Lierg6^{high} and Laerg6^{high} strains, including antiamastigote activity, toxicity, evaluation of the sterol profile, and molecular docking. **Supported by:**Cientistas do Nosso Estado - Faperj processo E26-201.158/2022 e Apoio às Instituições de Ensino e Pesquisa Sediadas no Estado do RJ- Faperj processo E26-210.157/2018 **Keywords:**Azasterols;Leishmania;24-C-methyltransferase.

PV-63 - **Extracellular vesicles from different virulence profiles Leishmania amazonensis plays a important role in parasite-parasite communication.**

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Leishmaniasis is a group of infectious diseases caused by *Leishmania spp*. It is estimated that up to 1 million new leishmaniasis cases occur every year, with 65,000 deaths occurring annually. Components expressed or secreted by *Leishmania sp.*, identified as virulence factors (VF), can participate in host's immune response subversion, playing a key role in infection success. *Leishmania spp.* can spontaneously release extracellular vesicles (EVs) containing parasite's antigens, VFs, RNA, DNA, and lipids. Data shows that EVs play a key role in the parasite-host relationship due to their ability to carry antigens and modulate the immune response. Studies shown that EVs can be a potential drug target for leishmaniasis treatment, as they also act by modulating the immune response, favoring the infection. Therefore, identifying its role in inter-parasite communication is of great interest. Thus, our objective was to verify VF genes expression in *In vivo*-derived (IVD-P (Virulent profile)) and Long-term *In vitro* culture (LT-P (Attenuated profile)) *L. amazonensis*, and perform EV transfer assays. For this purpose, both virulence profiles *L. amazonensis* were cultivated and RNAs were extracted for VF gene expression analysis using q-PCR. After, IVD-P and LT-P were cultured and EVs purified for transfer assays. EVs were stained with PKH-26 and promastigotes were analyzed by flow cytometry. Our results showed that IVD-P parasites had significantly increased LPG3 and GP63 genes expression when compared to LT-P. Transfer assays showed that IVD-P and LT-P parasites were able to internalize EVs originating from both IVD-P and LT-P strain. Also, confocal microscopy acquired images identified and certified the interaction between promastigotes and both IVD-P and LT-P EVs. In conclusion, *L. amazonenis* can modulate virulence genes expression depending on stimuli. Also, our study showed, in an unprecedented way, that *L. amazonensis* can communicate with adjacent parasites via EVs. **Supported by:**CNPq, CAPES, FAPESP **Keywords:**Leishmania amazonensis;Extracelular Vesicles;Transfer assay.

PV-64 - **Investigating the role of inositol pyrophosphates in cell survival, virulence, and maintenance of genomic integrity of *Leishmania braziliensis***

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Leishmaniases are a group of diseases caused by parasites of the genus *Leishmania* (Trypanosomatidae family). Currently, there are no vaccines or prophylactic measures, and existing drugs are toxic to the patients. In model eukaryotes, inositol pyrophosphates (PP-IPs), mainly IP_7 and IP_8 are involved in a wide range of cellular processes, such as regulation of telomere length and in the maintenance of virulence in some pathogenic fungi. The participation of IP6K and PP-IP5K kinases are essential in the IP_7 and IP_8 biosynthesis pathway, respectively. However, trypanosomatid parasites do not have orthologous genes for PP-IP5K, which makes them excellent models for studying the role of IP6K and consequently IP_7. In this study, lineages of *L. braziliensis* KO for IP6K ($IP6K^{-/+}$), telomerase ($TERT^{-/-}$) and double-KO ($IP6K^{-/+}$ + $TERT^{-/-}$) were generated using CRISPR/Cas9 approach to investigate the role of IP_7 in telomere homeostasis and its contribution to the virulence of this protozoan. The RT-PCR and RT-qPCR assays revealed the successful application of the CRISPR/Cas9 system in *L. braziliensis*. Although immunofluorescence assays (IFA) using α-γH2A revealed an accumulation of DNA damage in double-KO lineage ($IP6K^{-/+}$ + $TERT^{-/-}$), growth curves did not show a significant decline in their proliferation relative to the WT lineage. Moreover, DNA content analyses using flow cytometry did not indicate any cell cycle arrest. Currently, we are investigating where, in the genome, the DNA damage detected is occurring. Also, we are investigating possible morphological alterations due to the removal of IP6K. Subsequently, the lineages generated will be challenged with some genotoxic drugs to shorten telomeres (e.g.: H_2O_2), which will contribute to the understanding of the role of PP-IPs during telomere elongation in trypanosomatids. Of note, this work aims to shed light on the search for new potential molecular targets that can be used for the development new drugs against leishmaniases **Supported by:**FAPESP: 2022/00923-0
Keywords: Inositol Pyrophosphates;CRISPR/Cas9;Leishmania braziliensis.

PV-65 - **Pseudopod Projection and Modulation: Exploring Variability Across the Tree of Amoebozoan Testate Amoebae (Arcellinida: Amoebozoa)**

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Testate amoebae are unicellular eukaryotes provided with an outer covering (shell). These organisms protrude pseudopods through the single shell aperture and use them for locomotion, feeding, and other complex behaviors. The pseudopod is considered a key morphological character, and it is used to describe, distinguish, and classify amoeba groups. Despite the central role of pseudopods, the intra-specific morphological variability of this structure and the relationship between this variability and other features of the organisms has been poorly explored, including the correlation between this variability and different substrate types. In this study, we sampled representative species from two diverse infraorders of Arcellinida (Amoebozoa) that inhabit different natural environments and substrates. Using light microscopy, we observed their behavior on three different substrate types: agar plates, plastic bottles, and glass plates with liquid media. The primary objectives were to investigate the range of morphological variability in relation to organismal performance (i.e., locomotion speed), examine whether shell attributes and substrate types correlate with performance, and describe the nature of these correlations and their interplay with the variables. To achieve these objectives, we measured various features including shell diameter and area, pseudopod number per individual, and pseudopod length and width of multiple individuals. We then used these measurements and locomotion speed as variables in generalized linear models (GLMs) for further analysis, constructing separate models for each substrate type. The present study sheds ligth on the variability of pseudopod projection and modulation across the tree of amoebozoan testate amoebae (Arcellinida). Investigating pseudopod variability within Arcellinida leads to a better understanding of their diversity and differential capability to inhabit diverse habitats. **Supported by:**CAPES **Keywords:**cellular motility;microeukaryotic plasticity;variability.

PV-66 - **Consequences of IP6K disruption for Trypanosoma cruzi: the conundrum of inositol pyrophosphates pathway**

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Chagas disease is a neglected tropical disease caused by the protozoan parasite *Trypanosoma cruzi*. There are no effective vaccines and the available treatment options are effective only in the acute phase of the disease but can cause serious side effects on the patients. Thus, the search for the elucidation of molecular pathways that may provide potential targets for drug development is of paramount importance. Inositol pyrophosphates (PP-IPs) – mainly IP_7, and IP_8 – are involved in a wide range of processes in eukaryotes. However, the mechanism of action of these metabolites is not yet fully understood. IP_7 and IP_8 are synthesized by pathways involving the participation of IP6K and PP-IP5K kinases, respectively. Trypanosomatids have an ortholog gene for IP6K but do not have orthologs for PP-IP5K, making them excellent models for studying IP_7. Here, using CRISPR/Cas9 approach, we disrupted single and double alleles of IP6K, generating $IP6K^{-/+}$ and $IP6K^{-/-}$ lineages, respectively. IP6K inactivation causes several morphological effects, such as rounding and wrinkling of the cell body, increased number of glycosomes, and mitochondrial enlargement. Notably, $IP6K^{-/-}$ lineage (double-null) was unable to proliferate for more than 7 days, suggesting that this kinase is essential for this organism. Interestingly, $IP6K^{-/+}$ lineage (single-null) showed a slight cell cycle arrest at G0/G1 phase with no DNA damage (quiescent cells). Moreover, the $IP6K^{-/+}$ lineage showed a high proliferative capacity within the host mammalian cell, suggesting that the quiescent cells observed may be metacyclic forms. Together, our findings suggest that the total loss of IP6K has harmful consequences for *T. cruzi*. However, paradoxically, the disruption of a single allele of IP6K leads to *T. cruzi* being prone to multiplying further inside the host cell, generating a conundrum that we are still trying to understand. **Supported by:**FAPESP: 2020/16480-5
Keywords:CRISPR/Cas9;Inositol Pyrophosphates;Quiescence.

PV-67 - **Endosymbiosis in trypanosomatids: a study of the cell cycle in species of the *Kentomonas* genus**

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Kentomonas is a new genus of monoxenic trypanosomatid containing an endosymbiotic β-Proteobacterium that presents phylogenetic proximity to the symbiotic preparations present in *Angomonas* and *Strigomonas* genera. The relationship between prokaryotes and protozoan is mutualistic and characterized by intense metabolic exchanges, lost changes in associated partners, and bacterial division synchrony with other host cell structures. Considering these aspects, symbiosis in trypanosomatids represents an interesting model for evolutionary studies and also for comparison with heteroxenic trypanosomatids that are pathogenic to humans. This work aims to characterize the cell cycle of *K. deaneorum* and *K. sorsogonicus* and compare it with *A. deanei* and *S. culicis*. For that, we used growth curves, transmission electron microscopy (TEM), fluorescence microscopy (FM) and flow cytometry. Our results showed that the generation time of *K. deaneorum* is 4h, while that of *K. sorsogonicus* is 6h. TEM revealed that in the first species, the symbiont is closely associated with mitochondria, while in the second there is proximity between bacteria and glycosomes. An anti-porin antibody and DAPI revealed that the first structure to replicate is a symbiotic bacterium, which seems to use the nucleus as a topological reference during its division, followed by the kinetoplast and then the nucleus, as observed in *A. deanei* and *S. culicis*. We analyzed the cell cycle of both species by flow cytometry and established a protocol for synchronization using hydroxyurea, which caused an arrest in the G1 phase. In both species of *Kentomonas*, there is an adjustment of symbiont division along the host cell cycle, so that each generated cell has a single bacterium and a single copy of the nucleus and kinetoplast. Taken together, our results propose that trypanosomatids harboring symbionts represent an excellent model for studying the origin of organelles in the eukaryotic cell. Supported by FAPERJ and CNPq **Supported by:**161032/2021-4
Keywords:cell cycle;trypanosomatids;symbiosis.

PV-68 - **Optimization of Ultrastructural Expansion Microscopy (U-ExM) to study cytoskeletal structures and different organelles in *Trypanosoma cruzi***

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Ultrastructural Expansion Microscopy (U-ExM), is a method to improve the resolution of optical microscopes through the physical expansion of a sample. During U-ExM, a swellable polymer network (or hydrogel) is synthesized uniformly throughout a biological sample or tissue. The samples then undergo a process of mechanical softening and homogenization, followed by a process of isotropic three-dimensional physical expansion when water is added, causing the polymer and thus the sample in which the polymer is embedded in to enlarge. As a result, the biomolecules of interest become spatially separated from each other and the effective resolution of the microscope increases. This technique is compatible with standard immunofluorescence protocols.
We optimized an U-ExM protocol for *Trypanosoma cruzi* and quantifying the expansion factors of different subcellular compartments and organelles. We determine the localization patterns of different tubulin isoforms, such as α-tubulin and β-tubulin. Also we immunolocalized acetylated and tyrosinated α-tubulin isotypes in epimastigotes and use mitochondrial cell-permeable dyes to identify this organelle. Finally, U-ExM was also performed in trypomastigotes and amastigotes validating this technique in all life cycle stages of *T. cruzi*. We observed that the typical morphology of the epimastigotes is preserved during the process, achieving an expansion factor of 4.5 times. We analyzed the expansion of the nucleus, kinetoplast, and basal body to confirm the expansion by measuring their size with ImageJ software. These results allow us to corroborate the usefulness of this technique for *T. cruzi*, especially for the analysis of cytoskeletal proteins. Also, it is possible to appreciate within the nucleus areas with different fluorescence intensity, which allows distinguishing areas with different chromatin compaction without having to use electron microscopy or other super-resolution techniques. **Supported by:**Agencia Nacional de Promoción Cientifica y Tecnológica, Argentina (PICT2019-0526, PICT2021-0157), CONICET (PIBAA 1242)
Keywords: Expansion Microscopy;Cytoskeleton;Mitochondria.

PV-69 - **Single copy gene tagging as an alternative approach to study chromosomal aneuploidy in *Trypanosoma cruzi* by FISH**

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Recent studies have demonstrated considerable genomic variability and complex patterns of aneuploidy in *Trypanosoma cruzi*. However, the extent of variation and the classification of these aneuploidies into segmental or whole chromosome aneuploidy is still unknown. Here we investigated by Fluorescence In Situ Hybridization (FISH) the presence of aneuploidy in chromosome 24 (TcChr24) of *T. cruzi* (clone CL Brener) which presents evidence of trisomy by NGS. Initially, we analyzed the chromosomal ploidy of TcChr24 using as chromosome-specific markers three single copy genes mapped in this chromosome. It was possible to quantify the aneuploidy events in the populations from markers (TcCLB.503515.20, TcCLB.506413.70). However, It was not possible to estimate aneuploidy using the TcCLB.509127.80 as marker because it did not show sufficient labeling efficiency for quantification. Our data show that, for both genes, there is a higher percentage of monosomic cells, followed by disomic, trisomic and, rarely, polysomic cells. Then, we developed a new tool to analyze the aneuploidy events of the TcChr24, considering all three markers. We generated mutant parasites by CRISPR/Cas9, inserting an exogenous tag, in these three single copy genes, aiming to better understand the nature of the aneuploidy, whether whole or segmental chromosomal. Using the tag sequence as a probe, we achieved labeling efficiency above 85% for the three genes. Also, our data showed that most cells present monosomy, followed by disomy, trisomy, and polysomy for the three markers. This data corroborates what we found before in untagged cells, suggesting the occurrence of chromosomal somy variation for TcChr24. In conclusion, our data demonstrate the occurrence of non-homogeneously distributed aneuploidies in the population, that is, the subpopulations display cell-to-cell somy variation for a given chromosome, suggesting the occurrence of mosaic aneuploidy. **Supported by:**FAPESP (PD 2019/05049-4 and Thematic Project 2016/15000-4) and CNPq **Keywords:**CRISPR/Cas9;Aneuploidy;FISH.

PV-70 - **Exploring the Epidemiology, Genotypes, and Genetic Variability of *Toxoplasma gondii* in Sheep Populations of Uruguay**

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Sheep husbandry has been central to the economic and social development of Uruguay throughout history. Uruguay is currently the fifth largest exporter of sheep meat and third of combed wool in the world. However, reproductive indexes for the species is far from ideal. Our previous studies determined a high incidence of embryonic and fetal losses due to infection by *Toxoplasma gondii* whereby 58% of abortions of infectious etiology are due to this parasite). However, the prevalence of the parasite at the population level and the prevalent genotypes present in sheep flocks in Uruguay are unknown. Here, we determine the prevalent genotypes causing abortion in sheep by molecular and serological techniques. In addition, we isolated two novel strains from sheep (TgUru1 and TgUru2) and characterized them both genetically and phenotypically both *in vivo* and *in vitro*. Our findings suggest a broad genetic variability in the country, with atypical and unreported genetic types being predominant. Moreover, TgUru1 and 2 display radically distinct phenotypes, whereby the former is highly virulent and fast growing, whilst the latter is slow growing and displays high rates of spontaneous cystogenesis. We are currently analyzing the genetic and transcriptomic bases of these differences and their potential impact on the disease outcome. Additionally, we are exploring the correlation between our animal findings and the human population in the country in terms of the prevalent strain's genetic background. **Supported by:**Fondo Sectorial Salud Animal en ANII
Keywords: Sheep husbandry;Toxoplasma gondii;genotypes.

PV-71 - **Deciphering the role of YEATS domains in *Leishmania mexicana***

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Post-translational modifications (PTMs) govern crucial regulatory mechanisms in diverse organisms, influencing various biological processes. In 2011, lysine crotonylation, a novel PTM, was identified in histones. The crotonyl group, characterized by a rigid and flat C-C structure, has been found to modulate gene transcription and other vital cellular processes when added to the N-terminal chains of histones. The intricate regulation of crotonylation levels relies on crotonyltransferases, decrotonylases, and the readers, known as YEATS proteins. YEATS domains facilitate the binding of crotonylated lysines, facilitating the recruitment of regulatory proteins and triggering downstream effects. This study focuses on the characterization of YEATS domains in the protozoan parasite *Leishmania mexicana*. Utilizing global alignment analysis based on known YEATS domains from other organisms, we identified two YEATS-domain-containing proteins in *L. mexicana*, namely ENL and Yaf9. Protein structure modeling revealed conserved amino acids within the ß-strand 'sandwich' region, important for crotonyl group interaction. Furthermore, we observed distinct expression patterns of ENL and Yaf9 among parasite stages, suggesting their involvement in stage differentiation processes. To gain deeper insights into the biological functions of YEATS proteins in *L. mexicana*, we generated parasite overexpressing ENL (ENL-ox) and investigated their subcellular localization in the procyclic stage. Remarkably, we observed a stage-dependent differential nuclear localization pattern of ENL, with perinuclear distribution during non-dividing stages and dispersed nuclear localization during dividing stages. Moreover, a slight decrease in procyclic stage multiplication was observed in ENL-ox parasites compared to the wild-type cells. Future investigations will involve additional phenotypic analyses to comprehensively elucidate the role of crotonylation in *Leishmania* biology. **Supported by:**FAPESP 2021/04748-6
Keywords: Leishmania;Crotonylation;YEATS.

PV-72 - **Comparative molecular analysis of *Lutzomyia umbratilis*, the main vector of *Leishmania (viannia) guyanensis*, from endemic and non-endemic leishmaniasis regions of the Amazon state.**

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The epidemiological profile of the American Cutaneous Leishmaniasis in the Amazonian cities of Manacapuru (MNP) and Rio Preto da Eva (RPE), separated by the Negro River, differs, RPE being an endemic area of the disease and MNP showing no significant number of cases. With the aim of identifying molecular difference between the two populations, we performed midgut proteomic analysis . A total of 3292 proteins were identified, 3252 of which are shared by sandflies from both locations, 30 are exclusively present in MNP and 11 only in RPE. Among the proteins, eight were selected for transcriptional validation, based on the criteria of amount of reads and having a potential to impact on the vectorial capacity of insects. Of these eight proteins, four were only detected in MNP: SARA (Smad anchor for receptor activation), Citocromo P450, CysPc (Calcium-dependent cytoplasmic cysteine proteinases) and XPR1 (Xenotropic and Polytropic Retrovirus Receptor 1). The other four proteins were detected both in MNP and RPE, but they had a fold change greater than 2 or less than 0.5 in MNP: SL9B2 (Sodium/hydrogen exchanger 9B2), PATH (Proton Coupled Amino Acid Transporter Like Protein Pathetic), PEPT1-Like (Peptide Transporter 1-Like) and Paramiosin. With these targets defined, RT-qPCR was performed using insects from the two locations to compare the level of transcripts in carcasses and intestinal tracts. Our results showed that most of these proteins are upregulated in MNP, similar to the data from the proteomic analysis and are probably relevant in the mechanism of parasite transmission by the insect. We are now in the process of silencing some selected genes to analize their effect on infection. Using the proteomic data, we identified bacterial proteins and analized the microbiota composition of both populations, The possible implications of these data are being analysed in relation to the different vectorial capacity of these sand fly populations. **Supported by:**FAPERJ, INOVA-FIOCRUZ e IOC-FIOCRUZ
Keywords: Leishmania;Sand fly;Vectorial capacity.

PV-73 - **Effects of nalidixic acid on *Trypanosoma cruzi* evidences associated activity of kinetoplast proteins**

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Trypanosoma cruzi has a heteroxenic life cycle that presents structural variations during the cell differentiation process, which is known as metacyclogenesis. The non-infective epimastigote form is found in the intestine of the insect vector and is characterized by a disc-shaped kinetoplast containing a highly compacted mitochondrial DNA (kDNA). Topoisomerase II (topo II) and KAPs (Kinetoplast Associated Proteins) are essential enzymes to the kDNA replication, that involves the release of minicircles to the network. Nalidixic acid pertains to the quinolone group and is a potent topo II inhibitor, capable of blocking T. cruzi cell proliferation and promoting ultrastructural changes in the kinetoplast. In this work we used different methods, such as growth curves and analyzes by different microscopy techniques to verify if topo II inhibition differentially affects mutant cells of KAP7, a protein that has been related to kDNA repair mechanisms in this protozoan. For this purpose, we used WT DM28c cells, protozoa expressing KAP 7 fused to the fluorescent tag (mNG) and Myc, obtained by CRISPR-Cas9 and also protozoa with double knockout for KAP 7. The growth curves showed an inhibition of proliferation after treatment with the inhibitor in a concentration-dependent manner in the three cell types, especially in KO cells. Images obtained by transmission electron microscopy showed alterations in the kDNA arrangement, with the mutant strain being the most affected. Fluorescence microscopy showed the location of KAP 7 in the antipodal sites, the same place where topoisomerases II has been reported. The obtained data showed that the inhibition of topo II affects more strongly the cells with gene deletion for KAP7, indicating that these two enzymes have associated activities. However, further studies are necessary to determine if there is an interaction between KAP 7 and topoisomerase II. **Supported by:**PIBIC - UFRJ e Faperj **Keywords:**nalidixic acid;Kinetoplast Associated Proteins;Trypanosoma cruzi.

PV-74 - **Characterization of the Angomonas deanei microtubule cytoskeleton reveals new insights into symbiotic bacteria division**

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Some trypanosomatid species co-evolve with a symbiotic bacterium in a mutualistic relationship. Among such protozoa, Angomonas deanei stands out for presenting a better characterized genome, which allowed the development of tools for gene deletion. Previous analyzes of the host trypanosomatid cell cycle, showed a coordinated division between the bacteria and other cellular structures, resulting in the generation of daughter cells with a single symbiont. Studies using the RNAi system to modulate tubulin expression have shown that symbiont division is microtubule dependent. In this work, we used Trichostatin A (TSA), an inhibitor of histone deacetylases (HDACs) and also HDAC6 mutant cells obtained by CRISPR-Cas9 to better characterize the cytoskeleton of A. deanei and to verify whether the division of the symbiont is related to dynamic instability of microtubules. Treatment with TSA caused proliferation inhibition and affected the cell survival of mutant cells. The use of different optical and electron microscopy techniques showed morphological and ultrastructural alterations, both in TSA-treated cells and in mutant protozoa. Images obtained by fluorescence optical microscopy showed the filamentation of the symbiont, while those with negative stainning suggested changes in the distribution of microtubules. Cytometry data indicated cell cycle arrest in the G1 phase in TSA-treated cells, but not in HDAC6 mutant parasites. We conclude that the inhibition of deacetylation modifies structural aspects of the protozoan and is able to affect the division of the symbiont, suggesting that this event depends on the dynamism of the host microtubules.
Supported by:PIBIC-CNPq / 121774/2022-8 **Keywords:**symbiosis;trypanosomatids;division synchrony.

PV-75 - **Preliminary characterization of yeasts as potential symbionts of the mycobiota of the triatomine gut**

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In *Rhodnius prolixus*, a key vector of the Chagas's disease, shotgun metagenomic sequencing has unveiled a diverse range of bacteria involved in B-complex vitamin synthesis. However, the understanding of the fungal microbiota in triatomine insect remains limited. In this work, we aimed to characterize the diversity of the fungal gut microbiota of triatomines in order to identify yeast species known to establish symbiotic relationships with their hosts. We first analyzed the mycobiota associated with the digestive tract of triatomines captured in the field using two different approaches: amplification and sequencing of the D1/D2 26S rDNA from insect gut extracts, or axenic cultures of fungal species followed by morphological characterization and molecular identification of the isolates. A total of 90 fungal isolates were identified in the gut of 518 triatomines. Most of the identified species were classified as entomopathogenic, except for two yeast species of the genus *Aureobasidium* and *Trichosporon*. To investigate the nature of this association and its effects on insect physiology, *R. prolixus* first-stage nymphs were artificially fed with the two yeasts. We observed no impact of yeasts on mortality and insect physiology. Although yeast numbers decreased and became undetectable in insect homogenates by the fourth week after feeding, the presence of 26S rDNA amplicons persisted in the samples for a longer period after yeast infections. This suggests a possible association of the yeast with the insect tissues. We next investigated whether yeasts can use urate as their sole source of carbon and nitrogen, as urate is the end product of blood degradation in triatomine insects, found in high concentrations. We showed that both yeasts possess the ability to degrade urate. These preliminary results should contribute to a better understanding of the mycobiota and its role in the vector that could be used to develop strategies to control pathogen transmission by the vector. **Supported by:**CAPES **Keywords:**Mycobiota;Triatomines;Symbionts.

PV-76 - **Decoding the nuclear proteome of *Trypanosoma cruzi* across the cell cycle: insights into metabolic compartmentalization and epigenetics**

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The life cycle of *Trypanosoma cruzi* involves alternating forms between insect (replicative noninfective) and vertebrate (infective nonreplicative) hosts, posing challenges to parasite survival. This transition is accompanied by metabolic reprogramming from carbohydrates to amino acids as the primary energy source. Differentiation induces morphological alterations in the nucleus, including position, shape, and increased heterochromatin. These morphological changes are possibly linked to the role of histone post-translational modifications (PTMs) in chromatin organization, DNA replication, cell cycle and gene expression. Our main goal was to understand the dynamics of nuclear proteins and histone PTMs during the cell cycle using quantitative proteomic approaches. We synchronized the cell cycle with hydroxyurea, obtaining cells at G1/S, S, and G2/M. We isolated nuclei and histones and analysed the proteomes using LC-MS/MS with TMT labelling for nuclear proteins and untargeted methods for histone PTMs. We identified over 2,500 proteins, with 900 quantified within the nuclear proteome. Gene Ontology analysis was enriched in terms related to the "nucleus", "chromatin", and "chromosomes", supporting nuclear extract. Among the identified proteins,364 exhibited differential abundance throughout the cell cycle, including those involved in DNA and RNA processing and heat shock proteins (HSPs). Our preliminary findings indicated enrichment of proteins in metabolic pathways, such as "pyruvate metabolism," "tricarboxylic acid cycle (TCA)", "glucose/gluconeogenesis", and "L-histidine biosynthesis" at G1/S, suggesting potential metabolic compartmentalization in *T. cruzi* nucleus. The ongoing analysis aims to unravel the cell-cycle dynamics of nuclear metabolic proteins and explore possible correlations with histone PTMs. Understanding these mechanisms, we will provide valuable insights into *T.cruzi* cellular processes, survival strategies, and adaptive capabilities. **Supported by:**Fundação de Amparo a Pesquisa do Estado de São Paulo / **Keywords:**Trypanosoma cruzi;Cell cycle;Proteome.

PV-77 - **Quiescence in *Leishmania*, a target for a new generation of chemotherapeutic innovations**

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Quiescence is a reversible cell division arrest allowing cells to survive environmental insults and driven by a dynamic and regulated cell and metabolic remodeling program. Research on this essential adaptive skill in *Leishmania* is still in its infancy, yet it could explain many (sub-)clinical features of the disease. To characterize molecular mechanisms that control the emergence, and the dynamics of quiescence we developed in vitro models for dermotropic *L. braziliensis/L. lainsoni* and viscerotropic *L. donovani*. We developed and validated a quiescence biomarker (lower expression of GFP in rRNA locus) and observed a quiescent sub-population in cells exposed to stationary starvation, and this in both amastigote and promastigote life stages. In *L. lainsoni*, using bulk 'omic approaches, we found a dramatic decrease in the overall transcripts and low molecular weight metabolites in quiescent cells. However, specific genes and metabolites were relatively upregulated, for example autophagy-related genes, amastins, membrane-localized proteins and several polyhexoses and free fatty acids. In *L. donovani*, we used single cell RNAseq to monitor the modulation of transcriptome during the entire life cycle: in amastigotes, we discovered different sub-populations of quiescent cells, hereby highlighting the heterogeneity of the adaptive phenotype. We identified transcriptional markers of the respective sub-populations and we are currently functionally validating candidate drivers/regulators of quiescence. We also studied quiescence in the context of exposure to trivalent antimonials (PAT). Pre-existing quiescence provides drug tolerance and reciprocally, exposure to PAT can trigger quiescence, with an intensity varying among clinical isolates of a same species. Prospectively, our in vitro models could be used in R&D for untargeted screening of compound libraries, the identified drivers/regulators of quiescence could be targeted by specific inhibitors.
Supported by: FWO, Flemish Fund for Scientific Research **Keywords:** Palavras-chave: Quiescence;Drug tolerance;Single cell transcriptomics.