PV11 - **ROLE OF HISTONE-FOLD DOMAIN CONTAINING OOCYST RUPTURE PROTEINS DURING OOCYST AND SPOROZOITE STAGES**

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Sporozoites are produced in the mosquito inside the oocyst roughly 12 days after the uptake of an infected blood meal. Oocyst rupture is required for the release of the sporozoites, which next travel to the salivary glands where they will be transmitted to the new host. In Plasmodium two proteins containing histone-fold domains (HFD), which are usually found in DNA binding proteins, have essential roles in this process. We named these proteins ORPs (Oocyst Rupture Proteins) as mutant parasites lacking either one of the orps are unable to exit from the oocyst. Motile sporozoites develop normally but they remain trapped inside the intact oocyst capsule, leading to a complete block in transmission to mice. ORP1 is expressed in the cytoplasm of all Plasmodium stages and in oocysts it localizes to the oocyst wall. ORP2 is detected only in the cytoplasm of young oocysts and at the oocyst wall after sporozoites are formed. The HFDs of the two ORPs are directly implicated in the mechanism of oocyst rupture, possibly through the formation of a dimer. ORP1 and ORP2 have HFDs similar to subunits of the NF-Y transcription factor of higher eukaryotes but the parasite proteins are much bigger. Our recent data on progressive deletions of ORP2 show that other portions of ORP2 may play a role in the localization of the protein at the mature oocyst wall thus promoting the interaction of the HFDs of the two proteins and capsule rupture. Furthermore, mutant sporozoites lacking orp1 show a defect in gliding motility and invasion of liver cells and ORP1 is expressed in early liver stages suggesting a function of this protein also in these stages of the parasite. Our data show that Plasmodium exploited the DNA binding HFD for a divergent function in the unique process of oocyst wall rupture. ORPs, or their specific domains, could be a possible target for anti-malarial strategies development to stop malaria transmission to the vertebrate host. **Supported by: Keywords:** Oocyst; histone fold domain; anopheles

PV12 - **INVESTIGATING THE KENNEDY PATHWAY: PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE BIOSYNTHESIS IN *TRYPANOSOMA CRUZI*.**

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The Kennedy pathway is the main biosynthetic route for phosphatidylcholine (PC) and phosphatidylethanolamine (PE) formation. The importance of PC and PE synthesis in parasitic protozoa has been well demonstrated. Disruption of PC biosynthesis is fatal for *Plasmodium falciparum* and *Leishmania donovani*; disruption of PE biosynthesis is similarly fatal for *Trypanosoma brucei*. However, information is severely lacking with regards to phospholipid metabolism in *Trypanosoma cruzi*, the etiological agent of Chagas' disease.

Bioinformatic analysis of the *T. cruzi* genome suggest that unusually there is a single bifunctional choline and ethanolamine kinase (*Tc*CLB.508355.190), making it an attractive drug target as its inhibition would stop both PE and PC formation. This putative kinase (*Tc*E/CK) was recombinantly expressed in *Escherichia coli* and purified. Kinetic analysis revealed that *Tc*E/CK is bifunctional, with a higher affinity for ethanolamine than choline. Screening substrate analogues revealed *Tc*E/CK is promiscuous, tolerating substrates with tertiary amine substitutions and modifications to the carbon backbone. A compound library was screened to find potential inhibitors that could be translated into promising activity against *T. cruzi* epimastigotes in culture.

The subcellular localisation of *Tc*E/CK was determined to be cytosolic and possibly in the endoplasmic reticulum. Attempts to knock out a single allele of *Tc*E/CK through homologous recombination were unsuccessful, suggesting its essentiality. Therefore, sgRNA and replacement templates have been designed for CRISPR-cas9 gene editing. The lack of functional genes for compensatory PE/ PC biosynthesis suggests *T. cruzi,* like *T. brucei*, relies entirely on the Kennedy pathway, creating selectivity as humans have alternative routes for PE/PC biosynthesis. In conclusion, the enzymes of the Kennedy pathway in *T. cruzi* are appealing drug targets with a fundamental role in parasite survival and warrant further investigation. **Supported by: Keywords:** Phospholipid; lipid metabolism; t. cruzi

PV13 - **NUTRITIONAL STATUS AND SEROLOGICAL EVALUATION OF *TRYPANOSOMA CRUZI* INFECTION AMONGST DOGS FROM RURAL AREAS AT SOUTH OF ESPíRITO SANTO, BRAZIL.**

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Analysis of available information reveals that the emergence of *Trypanosoma cruzi* by oral transmission has an habitual character in the primitive endemic cycle of this parasite. Recent findings revealed in 2012 a first death by oral transmission of *T. cruzi* in Espírito Santo state, Brazil, notified in a child from a rural area of Guarapari. In this context, the present study proposes to evaluate the presence of *T. cruzi* infection amongst dogs from rural areas of Iconha and Alegre, both municipalities located at south of Espírito Santo, Brazil. Serological diagnosis for *T. cruzi* were performed in serum samples collected from dogs of households and peridomestic environment notified as triatomines infected by *T. cruzi* during the period of 2014 to 2017 by the Secretary of Sanitary Surveillance of Espírito Santo. Additionally, the nutritional status of dogs was evaluated according canine body mass index and body condition score. There were 36 triatomines from species *Triatoma vitticeps* and *Panstrongyllus geniculatus* previously notified as positive for *T. cruzi* in 31 households and two peridomestic environments, from which were evaluated thirty-three dogs. The anti-*T. cruzi* antibodies were detected in blood sample of nine dogs (27.27% of positivity). Canine body mass index revealed 15.15% of underweight, 33.33% of eutrophy, 48.48% of overweight and 2.77% of obesity. In turn, the body condition score system showed 27.27% of undernourished, 57.57% of eutrophy and 15.15% of supercharged (overweight, heavy, obese, grossly obese). Nutritional status was not associated with the presence of *T. cruzi* infection, according chi-square analysis. Our results indicated a new interesting epidemiological profile of *T. cruzi* endemic cycle at south of Espírito Santo state, since a considerable percentage of overweight instead of undernourished was observed among infected dogs considered as an important *T. cruzi* reservoir living nearby human beings. **Supported by:**CAPES; UFES
Keywords: Trypanosoma cruzi; serological diagnosis; canine nutritional status

PV14 - **TRITRYPDB, PLASMODB, VECTORBASE AND CLINEPIDB - GENOMIC, BIOINFORMATIC AND EPIDEMIOLOGIC TOOLS AND DATABASES TO FACILITATE RESEARCH**

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On behalf of The Eukaryotic Pathogen Database Resources and VectorBase, I present are a family of 13 area-specific, free, online genome and other Omics data mining resources that support almost 200 organisms within the protists and vectors as well as numerous oomycetes, fungi and several host species. These resources facilitate the discovery of meaningful biological relationships or testing of hypotheses from large volumes of integrated pre-analyzed Omics data with advanced search capabilities, data visualization and analysis tools. The graphic interface allows users to mine the data without the need for computational training. Data types include genome sequence, annotation, transcriptomics, proteomics, epigenomics, metabolomics, population resequencing, clinical data, and host-pathogen interactions. Data are analyzed using bioinformatics workflows and in-house analyses including domain predictions and orthology profiles across all genomes which permit inferences from data-rich organisms to organisms with limited or missing data. Our resources offers several perspectives for data mining – record pages compile all data for genes, pathways, etc; a genome browser for visualizing sequence data aligned to a reference genome; a search strategy system that queries pre-analyzed data and returns genes or features with shared biological characteristics; a private Galaxy workspace for analyses of user data and viewing in context with public data already integrated into EuPathDB. Our active user support offers an email help desk, social media, video tutorials and a worldwide program of workshops. These free resources easily merge evidence from diverse data and across species to place the power of bioinformatics with every scientist. Recent expansion includes ClinEpiDB.org, a site which facilitates the exploration and analysis of epidemiologic studies. Our future includes a merger with VectorBase, home to genome, Omic and population data for many vector species. **Supported by:**National Institutes of Health, Wellcome Trust, Bill & Melinda Gates Foundation **Keywords:** Data mining; data integration; analyses of user supplied data

PV15 - **TRYPANOSOMA CRUZI MEMBRANE TRANSPORTERS FOR DRUG DELIVERY AND AS DRUG TARGETS**

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Trypanosoma cruzi, the causative agent of Chagas disease, has a metabolism largely based on the consumption of glucose and amino acids. Among them, proline is also involved in differentiation processes, cellular invasion and stress responses. Polyamines are essential compounds to all living cells and in T. cruzi, besides their participation in cell growth and differentiation, its acquisition relies exclusively on transport processes since the parasite is unable to de novo synthesize them. In this work, computational simulations combined with in vitro assays were used to identify new inhibitors of the proline and polyamines transporters that also present trypanocidal activity. Crystal violet used to be applied in blood banks as a trypanocidal agent (discontinued due to its high toxicity) and it was selected for the similarity-based virtual screening as a starting point to find new inhibitors of the proline permease since its mechanism of action involves the inhibition of proline transport. To search for polyamine transport inhibitors, the reference molecule was a conjugate of a polyamine with anthracene, an experimental oncological drug. Using these compounds, a similarity screening was performed on structures databases of approved drugs. Three drugs were found to be in vitro inhibitors of the proline transporter and also had trypanocidal activity with IC50s between 1 and 13 µM in trypomastigote and amastigote forms. Other three drugs had similar effects over the polyamine transporter and the parasites with IC50s between 1 and 4 µM. The strategy herein applied, based on the screening of approved compounds used to treat other pathologies, is known as drug repurposing or drug repositioning. One of the main advantages of this experimental approach is that reduces the time and the economic cost of implementation of new therapeutic alternatives, which is especially important in neglected diseases, like Chagas. **Supported by:**ANPCyT - CONICET - Global Challenges Research Fund **Keywords:** Chagas disease; drug repositioning; nutrient transporters

PV16 - **LEISHMANIA AND GIARDIA FARNESYL DIPHOSPHATE SYNTHASE (FPPS), A BRANCH POINT OF THE STEROL METABOLISM.**

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The enzyme FPPS is in the intersection of several pathways of sterol metabolism, such as isoprenoids, dolichols, and ergosterol biosynthesis. *Giardia* is an ancient organism, which has lost the capacity to synthesize ergosterol, but FPPS is ubiquitous in eukaryotes, and inhibited by nitrogen containing bisphosphonates (N-BP). It was demonstrated activity of N-BP in parasitic protozoan but not investigated onto *L. infantum* and *Giardia*. Indeed, the mechanisms of cell death and damage in the ultrastructure caused by N-BP is unexplored. Thus, we evaluated the effect of N-BP, on the viability, ultrastructure, and performed phylogenetic analysis of FPPS comparing *Leishmania* and *Giardia*. As evaluated by the viability method risedronate displayed stronger anti proliferative activity in *Leishmania*, IC50 of 13.8 µM, followed by ibandronate and alendronate, which displayed IC50 of 85.1 µM and 112.2 µM. The effect of N-BPs was lower on trophozoites of G. duodenalis than in *Leishmania*, IC50 of 271 µM for ibandronate and 311 µM for risedronte. The ultrastructure of Giardeia treated with N-BP displayed concentric membranes around the nucleus and nuclear pyknosis. In Leishmania, were observed mitochondria swelling, myelin figures, double membranes, blebbs of the plasma membrane. Pointing to apoptosis, binding to Annexin-V, loss of mitochondrial membrane potential (TMRE) coupled to low percentage of *Leishmania* promastigores permeable to 7AAD. Multiple sequence alignment show that *Giardia* and *Leishmania* FPPS protein sequence display low identity, but display the conserved aspartate rich motif. *Giardia* and *Leishmania* FPPS are phylogenetically distant, but display conserved protein signature. The N-BPs effect, was more pronounced in *L. infantum* than *G. duodenalis*, which can relate to differences in the metabolism of these organisms, and to the differences in the FPPS expressed in each organism. **Supported by:**Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul **Keywords:** Leishmania; giardia; fpps

PV17 - **ENDOSYMBIOSIS IN TRYPANOSOMATIDS: THE BACTERIUM INFLUENCES *ANGOMONAS DEANEI* PROLIFERATION, ULTRASTRUCTURE AND METABOLISM OF CELLS GROWN IN DIFFERENT CARBON SOURCES**

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The co-evolution of *Angomonas deanei* with a symbiotic bacterium represents an important model to study the origin of organelles. There is an intense metabolic exchange between both partners and the symbiont favors the host protozoan oxidative metabolism. In this work, we investigated the symbiont influence on *A. deanei* proliferation, viability, ultrastructure and metabolism by comparing wild (AdWt) and aposymbiotic strains (AdApo) grown in different conditions: Warren complex medium, SDM80 containing glucose or proline, SDM80 without carbon source (fasting). Results showed that AdWt growth in SDM80 with glucose was 70% higher when compared to cells cultivated in proline for 36 h, whereas for AdApo this value corresponded to 32%, after 60h of cultivation. In both strains, 80% of cells were maintained viable in such conditions. Scanning Electron Microscopy (SEM) analysis showed that after fasting or in the presence of proline, cells became rounded, with a wrinkled surface and presenting shortening or absence of the flagellum. Observations by transmission electron microscopy (TEM) showed cells with mitochondrial alterations as swollen cristae. After growth in SDM80 with glucose cells presented a reduced flagellum and mitochondrial enlargement. A reduction of 24% in ATP production was observed in AdWt grown in SDM80 with glucose or in Warren medium, after KCN inhibition. However, this result was not repeated when cells were cultivated with proline or submitted to fasting. Conversely, AdApo did not show significant variation on ATP levels after growth on different conditions or inhibition by KCN. The AdWt O_2 consumption in cells cultivated with glucose was higher than that obtained with proline or submitted to fasting, whereas for AdApo no difference was observed for cells grown in such conditions. Data indicate that glucose is a better carbon source for the maintenance of *A. deanei* than proline and the symbiont presence enhance the metabolism of the host protozoan. **Supported by:**FAPERJ and CNPq **Keywords:** Endosymbiont harbouring trypanosomatids; cell growth and metabolism; carbon sources

PV18 - **MORPHOLOGICAL AND ULTRASTRUCTURAL CHARACTERIZATION OF NEW ISOLATES OF SYMBIONT-BEARING TRYPANOSOMATIDS PERTAINING TO *KENTOMONAS* GENUS**

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Trypanosomatids are well known for causing disease to men and to plants and animals of economic interest. However, most of them are non-pathogenic and inhabits an invertebrate host throughout their life cycle. Among these monoxenic there are species that co-evolve with a symbiotic bacterium in an obligatory relationship and are distributed in four genera: *Angomonas*, *Strigomonas*, *Kentomonas* and *Novymonas*. The symbiosis in trypanosomatids is marked by intense metabolic exchanges: most enzymes responsible for synthesis of heme are encoded on the bacterium that by the other side, benefits from the host phospholipid biosynthesis. The symbiont promotes ultrastructural changes in the host cell as in the paraflagellar structure, in microtubules distribution, in the kinetoplast shape and kDNA arrangement. In this work we characterized three new isolates of *Kentomonas* species, that were previously phylogenetically analyzed, by considering their morphology and ultrastructure. Transmission electron microscopy (MET) showed a close proximity of the bacterium to the mitochondrial branches in the isolate 2912 and fluorescence microscopy (FM) revealed that the symbiont was usually located in the anterior region. Interestingly, two isolates, 3062 and 3063, did not present the bacterium after analyses by PCR and microscopy methods. In all new isolates the kinetoplast displaces to the posterior end of the cell body, considering the nucleus as a topological reference, thus characterizing a cell differentiation event. Morphological analysis reveals that protozoa of the *Kentomonas* genus present quite varied dimensions considering the length and width of the cell body and the flagellum size. Recently, it was described that the *K. sorsogonicus* symbiont lost genes for the heme synthesis pathway. From these findings, we suggest that in the *Kentomonas* genus the symbiotic relationship has different characteristics when compared to those of *Angomonas*, *Strigomonas* and *Novymonas* genera. **Supported by:**FAPERJ and CNPq **Keywords:** Endosymbiont-bearing trypanosomatids; kentomonas genus; morphological and ultrastructural characterization

PV19 - **ANGOMONAS DEANEI AS A MODEL TO UNRAVEL THE FUNCTIONS OF KINETOPLAST ASSOCIATED PROTEINS**

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Angomonas deanei is a trypanosomatid that co-evolves in a mutualistic way with a symbiotic bacterium, that divides in synchronicity with other host cell structures. The trypanosomatid mitochondrial DNA is contained in the kinetoplast and is organized in a network composed by thousands of topologically interlocked DNA circles (kDNA). Its topological array varies according to species and stages of development and is related to the presence of histone like-proteins that associate to kDNA and are known as KAPs (Kinetoplast Associated Proteins). Such proteins neutralize the negatively charged kDNA, thus influencing the activity of mitochondrial enzymes involved in DNA replication, transcription and repair. In Angomonas deanei, a symbiont-bearing trypanosomatid, the kDNA fibers are disposed in a looser arrangement, thus facilitating the identification of kDNA topological alterations after using compounds that target KAPS. In this work we used the CRISPRCas9 system to delete the A. deanei kap4 or kap7 genes. The knocked-out cells were submitted to microscopy techniques to investigate possible changes on the kDNA arrangement, furthermore genotoxic agents as cisplatin, were tested to check effects on DNA damage and repair. Microscopy techniques revealed that KAP4 and KAP7 hemi-knockout cells, as well as KAP4 double knockout cells presented a high compactation of the kDNA network, cell division arrest and the symbiotic division impairment, that generated a filamentous bacterium. In such protozoa the kinetoplast division may not occur, generating cells with atypical number of structures. A. deanei knockout cells submitted to cisplatin treatment presented an exacerbated phenotypic modification, reduced proliferation and more damage in nuclear and kinetoplast DNA. Taken together, our data reinforces the idea that KAPs present essential roles in kDNA arrangement, replication and repair. Results also indicate that the symbiont division is coordinated to kinetoplast segregation. **Supported by:**CNPq and FAPERJ **Keywords:** Angomonas deanei; kdna; crisprcas9

PV20 - **THE ROLE OF SERINE AND THREONINE ON EPIMASTIGOTES BIOENERGETICS AND THEIR CAPACITY OF SUPPORTING THE PARASITE SURVIVAL UNDER STARVATION**

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Along its journey between mammalian and invertebrate hosts, Trypanosoma cruzi is subjected to dynamic environmental stress, such as severe nutritional stress (SNS), redox imbalance, temperature and osmolarity changes. In other organisms, Serine (Ser) and Threonine (Thr) have a critical role as carbon sources in SNS. In the T. cruzi genome database Ser and Thr biosynthesis pathway is absent. Nevertheless, Ser can be obtained reversibly from Glycine (Gly) through the Ser hydroxymethyl transferase. Ser and Thr can be converted into pyruvate or α-ketobutyrate, respectively, by a Ser/Thr dehydratase (S/TDH: 4.3.1.19). Then, these metabolites are capable to feed the TCA cycle. In this context, we decided to investigate the biological role of these amino acids in the parasite biology. Gly, Ser and Thr were incorporated from the extracellular medium by epimastigotes through a michaelian saturable transport system. The following kinetic parameters were obtained: Gly (K_M 0.902±0.007 mM and V_{max} 0.267±0.015 nmol Gly/min per $2x10^7$ cells); Ser (K_M 0.037±0.001 mM and V_{max} 0.258±0.02 nmol Ser/min per $2x10^7$ cells) and Thr (KM 0.087±0.006 mM and V_{max} 0.367±0.007 nmol Thr/min per $2x10^7$ cells). Furthermore, Gly, Ser and Thr were able to sustain viability and maintain proliferative capacity in SNS. Ser and Thr are decarboxylated in CO_2 up to 4h (5.53 ± 0.62 and 3.74 ± 0.52 $^{14}CO_2$ nmoles/$1x10^7$ cells), reestablish intracellular ATP levels and trigger O_2 consumption (Ser: 35.5 ± 4.2 and Thr: 36.2 ± 2.5 Slope pmol/(s*ml) O_2). The S/TDH activity was detected in epimastigote extracts. In addition, the putative TcS/TDH gene (systematic number: TcCLB.506825.70) was cloned and heterologously expressed which was used to produce specific antibodies allowing to determine its subcellular location as mitochondrial and cytoplasmic. Summarizing, we demonstrated here the participation of Ser and Thr in the resistance to starvation and the mitochondrial ATP synthesis. **Supported by:**FAPESP, CNPq **Keywords:** Starvation; aminoacids bioenergetics; trypanosoma cruzi

PV21 - **CHARACTERIZATION OF A POTENTIAL LINKER HISTONE IN TOXOPLASMA GONDII**

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The chromatin is a natural barrier to all DNA-dependent processes such as transcription. The chromatin compaction levels are regulated mainly by histones and their post-translational modifications (PTMs) that may act facilitating or preventing access to DNA. Toxoplasma gondii has the four canonical histones (H2A, H2B, H3 and H4), but so far, the fifth histone (H1 or linker histone), has not been identified. In other eukaryotes, H1 acts linking nucleosomes and its absence could interfere with the chromatin condensation. We identified a small and basic protein in Toxoplasma, similar to H1-like of bacteria, which we named TgH1-like. By immunofluorescence assay using the endogenous TgH1-like tagged, we found the protein located exclusively in the nucleus of tachyzoites. Performing standards histone extraction protocols, we observed TgH1-like in the same fraction that histone H4, which was confirmed by co-immunoprecipitation assays. Plaque assay experiments using TgH1-like knockout parasites showed a discrete but significant increase in the plaque number. In addition, replication assay showed an increase in the number of parasites per vacuole. Whereas in the invasion assay, it was observed an increase in the number of vacuoles per field. By immunofluorescence assay, it was observed in the knockout parasites, the presence of some vacuoles in an asynchronous replication, demonstrating a probable alteration in the parasite replication. Next, we investigate the nuclei architecture by electron transmission microscopy using ethanolic phosphotungstic acid staining, which allows to determine the localization of basic proteins such as histones and therefore chromatin. tgh1-like showed a different chromatin distribution compared to the control, suggesting TgH1-like has a role in the chromatin organization. To our knowledge this would be the first linker histone identified in Apicomplexa parasites and will provide new insights about the chromatin dynamics in Toxoplasma. **Supported by:**CAPES, CNPq, Fundação Araucária, Instituto Carlos Chagas - FIOCRUZ/PR **Keywords:** Toxoplasma gondii; chromatin; linker histone

PV22 - **BIOID APPROACH TO IDENTIFY PROTEIN INTERACTOMES OF POLY(A) BINDING PROTEINS IN TRYPANOSOMA BRUCEI**

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The fates of mRNA transcripts are in part regulated by RNA-binding proteins (RBPs), which guide these RNAs through synthesis, storage and degradation. Poly(A)-binding proteins (PABPs) control mRNA stability and translation by interactions with the poly(A) tail and translation initiation factor complex eIF4F. Trypanosomatids have two PABP isoforms, PABP1 and PABP2. By affinity purification of cryomilled procyclic cells we found major differences in the interacting proteins between the isoforms, indicative of different mRNA targets (Zoltner, et al, 2018). Here, we have extended these studies by a novel BioID method based on the biotin ligase TurboID (Branon, et al., 2018). TurboID is targeted by genetic fusion to each PABP and causes biotinylation of all proteins within a few nanometers. The biotinylated proteins are isolated with streptavidin coated-beads and analyzed by mass spectrometry. Importantly, fusion proteins are expressed from their native loci at endogenous expression level, avoiding artefacts from overexpression. We will compare the PABP interactomes obtained by cryomilling affinity purification and BioID, to validate the novel BioID method, but also to investigate differences between the methods. The major advantage of BioID is that it only detects in vivo interactions, while affinity purification can identify interactions that occur in the lysate (false positives). Preliminary data show that the BioID method using the biotin ligase TurboID is functional in trypanosomes. The next steps are to compare protein partners of PABP1 and PABP2 between different life cycle stages. We are also testing the method with several other T. brucei RNA binding proteins. The identification of protein interactions is of major importance for most research questions and we here extend the set of available methods for trypanosomes. **Supported by:**CAPES/DAAD **Keywords:** Turbo-id; trypanosoma; pabp

PV23 - **DNA MISMATCH REPAIR PROTEINS ACT DIFFERENTLY IN RESPONSE TO DNA DAMAGE CAUSED BY OXIDATIVE STRESS IN TRYPANOSOMATIDS**

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MSH2 and MSH6 are central components of the eukaryotic DNA Mismatch Repair (MMR) pathway responsible for the recognition and correction of base mismatches that occur during DNA replication and recombination. Previous studies from our group showed that *Trypanosoma cruzi* and *Trypanosoma brucei* MSH2 play an additional role in DNA repair in response to oxidative damage. Here we investigated the involvement of other MMR components in the oxidative stress response by analyzing knockout mutants of MSH6 and MSH3 that were generated in *T. brucei* bloodstream forms as well as MSH6 *T. cruzi* epimastigote knockouts created using CRISPR/Cas9. Differently from the phenotype observed with *T. cruzi* MSH2 knockouts, loss of one or two alleles of MSH6 in *T. cruzi* resulted in increased susceptibility to H_2O_2 exposure, but not in impaired MMR as no difference in tolerance to alkylation by MNNG was observed in MSH6 mutants compared to wild type parasites. Unlike MSH3 knockouts, *T. brucei* MSH6 null mutants displayed increased tolerance to MNNG treatment, suggesting that MSH6 is directly involved with mismatch repair. However, no difference in the oxidative stress response was observed when *T. brucei* MSH6 mutants and wild type bloodstream forms were submitted to H2O2 treatment. Taken together, our results suggested that, in addition of being an essential component of the MMR pathway in *T. cruzi* and *T. brucei*, MSH2 also acts in the oxidative stress response in both parasites. In contrast, while *T. cruzi* MSH6 may be also involved with MMR, it has an additional role in the oxidative stress response through an yet poorly characterized new DNA repair pathway that operates differently in *T. brucei* and *T. cruzi*. Because these two members of the trypanosomatidae family are human pathogens well adapt to distinct host environments, in which oxidative stress is always present, further studies on DNA repair mechanisms and their interconnections with other cellular pathways are urgently required.
Supported by: Capes, CNPq **Keywords:** Trypanosoma cruzi ; dna mismatch repair; oxidative stress response

PV24 - **GENETIC POLYMORPHISM OF PLASMODIUM VIVAX METACASPASE-1 IN ISOLATES FROM BRAZILIAN AMAZON**

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The current emergence and spread of drug-resist[illegible]malaria control in the world and, in this context, elucidation [illegible]d provide alternative molecular targets to the development [illegible]at protozoan can trigger programmed cell death (MCP) pr[illegible]o be mediated by a family of proteases called metacaspas[illegible]MCP was not investigated yet, but studies in endemic area[illegible]metacaspase 1 (PvMCA1) gene presents both size and sing[illegible]ypothetically be involved in drug resistance. In the present w[illegible]MCA1, resulting from 30-nucleotide tandem repeats, in 37 P[illegible]Mâncio Lima, Acre State. Analysis of the repeat region of Pv[illegible]ragments of distinct size corresponding to 450pb and 500pb, wh[illegible]ragment (81%). Size polymorphism was confirmed by sequencing, whi[illegible]haracterized by presence of one (TR1) or two (TR2) tandem repeats. TR1 allele[illegible]t and presented nucleotide sequence similar to the reference Sal-1 strain, while in TR2[illegible]of adenine for guanine in the second repeat unit was observed, as already described in othe[illegible]endemic areas. The data indicates a limited diversity of PvMCA1 gene concerning its repeat re[illegible]on, whose relation to parasite susceptibility/resistance profile to antimalarial drugs is still to be established.
Supported by:CNPq e FAPERJ **Keywords:** P. vivax; genetic diversity; metacaspase

PV25 - **WHAT MAKES US DIFFERENT? : GENERATION OF *TRYPANOSOMA BRUCEI* CELL LINES EXPRESSING *TRYPANOSOMA CRUZI*'S HIS DEGRADATION PATHWAY**

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Trypanosoma cruzi is the etiologic agent of Chagas disease, which is transmitted by triatomine insects. As the life cycle of *T. cruzi* needs an invertebrate vector and a vertebrate host to completion, this parasite is highly adapted to different environments. For example, when glucose is limited in the insect vector's gut, *T. cruzi* can degrade amino acids producing ammonia. In this context, histidine (His) can be found in high concentrations in the insect's gut and it can be used as an ATP source by the parasite. Additionally, *T. cruzi* can completely oxidise His and use it to restore cell viability after long-term starvation, as it has been previously observed in our lab. The His degradation pathway consists of four enzymatic steps and putative coding sequences for all the enzymes are present in the *T. cruzi*'s genome. However, they are absent in *Trypanosoma brucei* what makes it a great model to investigate the biological role of this pathway. In order to express the complete putative His degradation pathway from *T. cruzi* in *T. brucei*, we developed a series of plasmids, named pJG. We transfected *T. brucei* procyclic form with the plasmid pJG001, which allows constitutive expression of histidine ammonia-lyase from *T. cruzi* (*Tc*HAL). There is no difference in proliferation between the wild-type control and transfected parasites and Western blotting shows successful expression of *Tc*HAL. Measurement of the *Tc*HAL enzymatic activity in cell extracts shows that *T. brucei* expressing *Tc*HAL is capable of degrading histidine but with lower activity when compared with *T. cruzi* epimastigotes. Immunofluorescence shows that *Tc*HAL is localised mostly in the cytoplasm of *T. brucei,* similarly to *T. cruzi.* Analysis of the complete His degradation pathway in *T. brucei* can contribute to elucidate which evolutionary advantages it brings to *T. cruzi* life cycle and provide valuable insights to the development of drugs that interfere with its metabolism. **Supported by:**FAPESP **Keywords:** Trypanosoma cruzi; trypanosoma brucei; metabolism

PV26 - **CHARACTERIZATION OF ZINC-DEPENDENT LYSINE DEACETYLASES OF *LEISHMANIA***

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Protein lysine acetylation plays a relevant role in the regulation of essential cellular processes in different organisms. Lysine acetylation levels are regulated by the activity of two families of enzymes: lysine acetyltransferases (KATs) and lysine deacetylases (KDACs). The KDACs are divided into two classes: zinc-dependent and NAD+-dependent, or sirtuins, lysine deacetylases. In general, Trypanosomatids has four and three genes in their genome coding for zinc-dependent and sirtuins KDACs, respectively. Recently, our group characterized the acetylome of *Trypanosoma cruzi* and *Trypanosoma brucei*, and observed hundreds of proteins related to essential cellular processes acetylated, suggesting that protein acetylation play important role in the biology of these parasites. Thus, to better understand the function of this modification in Trypanosomatids, we decided to characterize the four zinc-dependent lysine deacetylases of *Leishmania mexicana*. First, using the CRISPR-Cas9 system we obtained knockouts and tagged parasite cell lines for these proteins. We were able to generated knockout cell lines for DAC1, DAC4 and DAC5, but not for DAC3 protein. On the other hand, we successfully obtained tagged strains for DAC3 and 4, and found that both proteins are nuclear. We are working in the DAC1 and 5 tagged cells. Using these cell lines, we evaluated the effect of DACs in the parasite multiplication and differentiation. The DAC3 and DAC5 knockouts affects significantly promastigote replication; while no difference was observed in the tagged parasites. Also, DAC3 single-knockouts have an impaired differentiation to metacyclic-promatigotes forms. Experiments are under way to characterize the effect of the other DACs in these processes, but these preliminary data suggest that DACs are important in the regulation of these essential processes in *Leishmania*, opening the opportunity to explore these proteins as drug targets in these parasites. **Supported by:**FASPESP **Keywords:** Leishmania mexicana; acetylation ; deacetylases

PV27 - **MODULATION OF *TRYPANOSOMA CRUZI* AMPK PHOSPHORYLATION DURING EPIMASTIGOTES GROWTH**

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Trypanosoma cruzi has a high ability to adapt to the nutritional environment, but the mechanisms that signal this adaptation have not yet been fully elucidated. Control of the energy status of eukaryotic cells occurs through AMPK, a protein kinase activated as a consequence of the increase in AMP over ATP levels, which coordinates cell growth, autophagy and metabolism. It has recently been described that AMPK may also be activated by decreasing glucose levels regardless of the AMP / ATP ratio in trypanosomes. Here we examined the AMPK phosphorylation levels in the threonine 172 of the α1 catalytic subunit of this enzyme. We have found that this residue is dephosphorylated in exponentially growing epimastigotes upon dilution in fresh medium, irrespective of the presence or absence of glucose. AMPK phosphorylation was reestablished progressively, reaching the original levels at 24 hours of incubation. The same occurs in saline solution but the phosphorylation levels became higher than in rich medium. Addition of dorsomorphin, a general inhibitor of AMPK prevents growth in rich medium but no change in the AMPK phosphorylation was observed either in glucose rich or poor conditions. In contrast, in saline solution no increase in the phosphorylation occurs, ATP levels decrease, the parasites become round and lose the flagellum. These results suggest that phosphorylation could be related to AMPK multiple roles in parasite adaptation to distinct conditions, which are not necessarily dependent on glucose levels. **Supported by:**Fapesp **Keywords:** Ampk; glucose; metabolism

PV28 - **STUDY OF PHOSPHOLIPASE A2 IN *RHODNIUS PROLIXUS* MIDGUT**

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Rhodnius prolixus is a hematophagous insect of the Hemiptera order, Triatominae subfamily and is a known vector of a parasite named *Trypanosoma cruzi*, etiologic agent of Chagas's disease. Fighting against the vector is still the best way to prevent this disease and, because of that, finding molecules that are crucial for the infection is necessary. In this scenario, previous studies from our group demonstrated the involvement of a lysophospholid, lysophashatidylcholine (LPC), in different aspects of the infection. Thus, the aim of this work was to study a superfamily of enzymes that can promote the release of this lysophospholid, phospholipases A_2. In the present study, phospholipasic A_2 activity of different classes of PLA_2 from the lumen and epithelium of anterior and posterior midgut was analyzed days after insect's feeding using enzymatic assay. In anterior and posterior midguts it was possible to observe activity of secreted PLA_2 ($sPLA_2$) and platelet activating factor acetylhydrolases (PAF-AH), whereas calcium independent PLA_2 ($iPLA_2$) activity was only detected in the anterior midgut epithelium and posterior midgut lumen. To verify the possible roles of two genes of $sPLA_2$, $RpPLA_2$ 4037 and 9995, insects were injected with dsRNA for silencing and feeding performance was analyzed. Silenced insects ingested significantly less blood, but the phospholipasic A_2 activity in the midguts showed no statistically difference. When the gene expression of other $sPLA_2$ were analyzed, there was a trend of increase expression of the gene $RpPLA_2$ 8617 in anterior midgut and $RpPLA_2$ 8619 in posterior midgut when the gene $RpPLA_2$ 4037 was silenced, while when $RpPLA_2$ 9995 gene was silenced, the expression of $RpPLA_2$ 8619 gene tends to increase in the anterior midgut and is significantly higher in posterior midgut, showing a compensating effect. Thus, this work contributes to a better understanding of the role of different PLA_2 classes for the vector *Rhodnius prolixus*. **Supported by:**CAPES **Keywords:** Rhodnius prolixus; phospholipase a2; digestion

PV29 - **EXPRESSION, PURIFICATION AND CHARACTERIZATION OF THE AMINO TERMINAL FRAGMENT OF THE *LEISHMANIA SPP*. TELOMERASE REVERSE TRANSCRIPTASE (TERT) COMPONENT.**

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Leishmaniasis is a neglected tropical disease that presents different clinical forms and affects de most impoverished populations in Africa, Asia, and Latin America, especially Brazil. It is caused by a protozoan parasite belonging to the genus *Leishmania*, which is transmitted by the bite of a sandfly. Finding therapeutic targets for the development of new drugs is indispensable, once the available anti-parasite therapies have low efficacy, high toxicity and high cost and can induce parasite resistance. Telomerase is the enzyme responsible for replicating telomeres, the physical ends of eukaryotic chromosomes, which are vital to maintaining parasite's life cycle and reproduction within the hosts. TERT is the protein component of telomerase, which presents at it's amino-terminal two structural and functional domains, TEN and TRBD, involved with enzyme catalysis and association with the telomeric DNA and with the RNA component. Understanding these associations is pivotal for the development of new anti-parasite therapies. Our goal was to express and purify the aminoterminal fragment of the Leishmania amazonensis TERT component (LaTERT n-term), to perform protein-nucleic acid interaction assays. The recombinant protein was obtained using a bacterial heterologous expression system and different *Escherichia coli* strains as hosts. To find the ideal conditions of protein expression, we also tested different concentrations of IPTG different expression time and temperature. The results show that the protein is expressed both in *E. coli* BL21(DE3) códon plus RP with 1 mM IPTG for 4 hours at 37 °C and in *E. coli* Artic Express (DE3) Ril with 1 mM IPTG for 4 hours at 30ºC. The solubility tests demonstrated that the proteins were expressed in inclusion bodies or the insoluble portion of the protein extract. We are standardizing the purification protocol by isolating the protein from the bacterial inclusion bodies. The refolded protein will be tested using biophysical assays. **Supported by:**FAPESP **Keywords:** Leishmania; telomeres; telomerase

PV30 - **DEVELOPMENT OF *PLASMODIUM VIVAX* ASEXUAL BLOOD-STAGE IS INHIBITED BY PROTEIN KINASE INHIBITOR STAUROSPORINE**

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Despite impact of Plasmodium vivax in global pub[illegible] knowledge regarding its biology, which in some extent can[illegible] for vivax malaria urgently needed due to current emergenc[illegible]. While cell signaling of Plasmodium parasites is still far to b[illegible]own that protein kinases play a central role in blood-stage dev[illegible]udies evaluating kinase activity on P. vivax. In the present st[illegible]cts of staurosporine, a broad-spectrum protein kinase inhibit[illegible]ected erythrocytes were enriched from blood sample of P. vi[illegible]sence of staurosporine. Parasite maturation was determine[illegible]ected erythrocytes in Giemsa stained blood smears as well a[illegible]n flow cytometry assays. Additionally, parasite viability and h[illegible]w cytometry using rhodamine 123 and annexin-V, respectively. Tre[illegible]eased significantly the number of schizont-infected erythrocytes when com[illegible]parasites, as also evidenced by a reduction of parasite DNA content detected using [illegible]. Furthermore, loss of parasite mitochondrial membrane potential as well as increas[illegible]e of phosphatidylserine on parasitized erythrocyte surface were observed in staurosp[illegible]-treated parasites. Our data indicate a key role of protein kinases in P. vivax maturation, whose inhibition of activity leads to parasite death that, in turns, induces eryptosis of parasitized erythrocyte. **Supported by:**FAPERJ, CNPq **Keywords:** P. vivax; protein kinase; cell death

PV31 - **TGHDAC4: A HISTONE DEACETYLASE UNIQUE TO APICOMPLEXA.**

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Toxoplasma gondii is an obligate intracellular parasite member of the phylum Apicomplexa and is responsible for toxoplasmosis, a disease that affects a quarter of the world's population and which has no effective cure. Epigenetic regulation is one of the mechanisms controlling gene expression that seems to play an important role in this parasite. A category of enzymes that act as epigenetic regulators are the histone deacetylases (HDACs), which have a function associated with gene silencing in other eukaryotes. *Toxoplasma* has seven HDCAs and we are particularly focused in TgHDAC4, an enzyme unique of Apicomplexa parasites and a class IV HDAC, the less characterized class of deacetylases. Database searches showed that the only region shared with other organisms is a portion of the typical HDAC domain. To explore the function of TgHDAC4, we constructed an endogenous tag line (TgHDAC4-HA). By indirect immunofluorescence and co-localization assays, we verified that TgHDAC4 is located in the apicoplast, and co-localized with TgHU, a histone-like protein from prokaryotes. Preliminary data of transmission electron microscopy, confirmed the presence of TgHDAC4 at the apicoplast, in the periphery of the organelle. Western blot assays identified a protein smaller than expected, of about 100 kDa, common in apicoplast proteins due to cleavage signal and transit peptides. We added a YFP tag on the predicted signals and preliminary data showed a similar label at apicoplast. In addition, we performed an immunoprecipitation assay followed by mass spectrometry and identify some apicoplast proteins which are possibly targets of TgHDAC4. Phylogenetic analysis from TgHDAC4 domain shows a possible prokaryotic origin. Understanding how this protein works could provide new insights into the metabolism of the apicoplast, an organelle essential for parasite survival. **Supported by:**CAPES, CNPq, Fundação Araucária, Fiocruz **Keywords:** Toxoplasma; histone deacetylase; apicoplast

PV32 - **TRYPANOSOMA EVANSI SECRETOME AS A STRATEGY FOR PROTEIN MARKER IDENTIFICATION**

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Trypanosoma evansi is the causative agent of Surra, a disease that causes stock around the world. It is an African parasite with close genetic similarity to al of this work was to reveal the T. evansi secretome focusing on identi tic and therapeutic biomarkers. For that purpose, the parasites purified on DEAE-Cellulose, maintained during 2h at 37°C and ed proteins were measured by NanoDrop®, separated by S pectrometry. The average concentration was 0.98 µL/mL (37°C oteins were identified having an average molecular mass of e bioinformatics software predicted 31 proteins containing a aving a transmembrane domain (TMHMM/Phobius), 104 ow ic reticulum (ERRet), 3 having GPI-anchorage (PredGPI) cretion (SecretomeP). Most proteins were predicted as cyto RT (266) and ProtComp (279) and a few as extracellular . It was predicted 3,823 non-N-glycosylated distinct epitope 514 epitopes are unique to T. evansi when compared to T and T. equiperdum. The identified proteins were clustered in 371 o gambiense, T. vivax, and T. equiperdum, however, 2 paralog groups cor ingleton proteins were detected only in T. evansi. Our finds indicate that temp protein secretion (p = 4.98E-11) and suggest that 32 proteins and 514 epitopes found argets for the differentiated diagnosis and treatment of Surra. Finally, this research was the escribe the T. evansi secreto-me **Supported by:**CNPq e UDESC-FAPESC **Keywords:** Surra; b nformatic; proteome

PV33 - **CHARACTERIZATION OF THE *TRYPANOSOMA CRUZI* TCK2, AN EIF2 PROTEIN KINASE AS A POSSIBLE DRUG TARGET**

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Eukaryotic translation initiation factor 2 kinases are enzymes that phosphorylate eIF2α under stress situations to generate remedial responses. *Trypanosoma cruzi* has three eIF2 related kinases (TcK1-3). TcK2 is topologically related to mammalian PERK and was found relevant for infection and parasite growth. To understand its role during parasite infection and proliferation in mammalian cells, soluble recombinants with the catalytic domain of the enzyme were expressed in *E. coli*. Antibodies were produced and showed that TcK2 is more expressed in tissue culture trypomastigotes and intracellular amastigotes compared to metacyclics and epimastigotes respectively. We also generated a new TcK2 gene knockout by CRISPR/Cas9 inserting a stop codon at the position 34 of the gene. About 90% of epimastigotes with the mutation showed no detectable TcK2 by Western blots. TcK2KO cells presented a slow grow phenotype in epimastigotes as previously found. TcK2KO metacyclics infected mammalian cells but after successive infections produced trypomastigotes without the stop codon mutation confirming its requirement for parasite growth in mammalian cells. In parallel, we found that only some recombinants presented activity in a TR-FRET energy transfer assay (LANCE® Ultra Kinase Assay) using CREBtide (Perkin Elmer) and p70S6K, recognized PERK substrates, but not Histone H3. The active enzyme encompassed residues S659-E891 of the full TcK2 and deletion of 2 Carboxy-terminal amino acids of the predicted P-loop (P764-D773) increased the activity, whereas removal of 4 and 8 amino acids inactivated the enzyme for the two tested substrates. Further characterization of TcK2 functions for the parasites complemented with identification of specific inhibitors and ongoing crystallization assays might enable us to develop therapeutic target for Chagas' disease treatment. **Supported by:**CNPq **Keywords:** Tck2; trypanosoma cruzi; inhibitors

PV34 - **AN OVERLOOKED IMPORTANT POST-TRANSLATIONAL MODIFICATION: THE BEGINNING OF AN EVOLUTIONARY STUDY ON INOSITOL PYROPHOSPHATES AND ITS ROLE IN KINETOPLASTID DNA METABOLISM**

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Inositol pyrophosphates (PP-IPs) are a specialized group of molecules involved in various cellular processes, such as DNA metabolism. However, the function of these molecules is not yet fully elucidated. The best-studied PP-IPs are IP7 and IP8, which are synthesized by IP6K and PP-IP5K kinases, respectively. Pyrophosphorylation by PP-IPs is a non-enzymatic process and represents an important form of a post-translational modification. So, my group is interested in understand the role of the PP-IPs in DNA metabolism using kinetoplastids as a model. The preliminary data here suggest that trypanosomatids (T. cruzi and L. major) have orthologs for IP6K, but they have no orthologs for PP-IP5K, which prevents them from synthesizing IP8, suggesting that IP7 is the major PP-IP in these organisms. Interestingly, the free-living organism Bodo saltans (a kinetoplastid closely related to the trypanosomatids) has ortholog candidates for both PP-IP5K and IP6K, suggesting that the loss of PP-IP5K in trypanosomatids occurred during the transition from a free-living to a parasitic lifestyle. Whether IP7 fulfills some function in trypanosomatid that is exercised by IP8 in Bodo saltans is an open question that needs further investigation. Moreover, the C-terminal functional domain from IP6K ortholog in trypanosomatids is conserved and resembles the functional domain of IPK2 from S. cerevisiae. Also, IP6K has no paralogs and it appears as a single copy gene in the trypanosomatids genome. This feature will facilitate future approach aiming the depletion of IP6K in order to investigate further the role of IP7. Besides, assays using labeled IP7 to track its targets are being performed and will shed light on the mechanism of action of these metabolites. Together, these preliminary data point out that this work has great potential to explain the diversification and function of PP-IPs, not only in kinetoplastids but also in any cell that needs pyrophosphorylation to maintain DNA homeostasis.
Supported by: Keywords: Inositol pyrophosphates; dna metabolism; post-translational modification

PV35 - **NUCLEOSOME OCCUPANCY MIGHT CONTRIBUTE FOR THE RECRUITMENT OF PRE-RC ONTO DNA IN *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi is the etiological agent of Chagas disease. During the life cycle, *T cruzi* has distinct cellular forms that are either able to divide (epimastigote and amastigote) or non-replicative cells able to actively infect cells (trypomastigote). The DNA replication in trypanosomatids has gained more attention due to the wide divergence in how these organisms deal with the duplication of the genome. Eukaryotic DNA replication initiates from multiple distinct sites in the genome, termed origins of replication. Prior to S phase, multiple origins are poised to initiate replication by recruitment of the pre-replicative complex (pre-RC). In *T cruzi*, we have previously showed that pre-RC is not recruited onto DNA in non-replicative forms. For proper replication to occur, origin activation must be tightly regulated and many studies have shown that chromatin can strongly influence initiation of DNA replication. Our data showed that when we immunoprecipitated Orc1/Cdc6, a component of the Trypanosoma pre-replication machinery, we found histone H3 and H4, suggesting a correlation between origins of replication and the nucleosome positioning. To analyze the nucleosome occupancy, we generated genome-wide maps of nucleosome positioning by MNase digestion coupled to next-generation sequencing (MNase-seq) from replicative and non-replicative forms. We next sought to determine whether nucleosome occupancy correlates with origins. Collected data were processed using the Monte Carlo method to simulate the correlation; 1,000 iterations were used in the simulation model, and showed predominance of origins around nucleosomes. However, our data suggested that nucleosomal occupancy is similar in replicative and non-replicative forms. Therefore, we concluded that although nucleosome occupancy might contribute for the recruitment of pre-RC onto DNA, it might not be involved with the lacking of Orc1/Cdc6 recruitment in the non-replicative forms.**Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) **Keywords:** Nucleosome occupancy; trypanosoma cruzi; pre-replicative complex

PV36 - **ORIGIN OF THE LEISHMANIA LINEAGE: RECONSTRUCTING ANCESTRAL AREAS**

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Three hypotheses exist on the origins of *Leishmania* lineage: the Palearctic hypothesis states an origin in the Palearctic region during Cretaceous, with reptiles as their original vertebrate hosts, dispersing via terrestrial bridges to the Nearctic and Neotropical regions; the Supercontinent hypothesis proposes an origin prior to the Gondwana break up, with mammals has their first vertebrate host, and vicariance events playing a major role in the lineage distribution; the Neotropical hypothesis places the origin of *Leishmania* in the Neotropical region also stating mammals has their first vertebrate host. The aim of this work is to discuss historical processes that may have influenced current patterns of geographical distribution of *Leishmania* based on hypotheses of phylogenetic relationships of the genus and methods of reconstructing ancestral distributional areas. Gene sequences from gGAPDH (811bp), HSP70 (636bp) and V7V8 SSUrRNA (790bp) were retrieved from GenBank (NCBI) and TriTrypDB databases in a total of 39 terminal taxa (11 from *Leishmania*). Sequences were aligned on ClustalW implemented on MEGA7, most suitable evolutionary models were accessed on jModelTest and Bayesian analyses were performed on MrBayes 3.2.7. Reconstruction of ancestral areas was performed on RASP 4.2 using a Statistical Dispersal-Vicariance analysis (S-DIVA) and a Dispersal-Extinction Cladogenesis analysis (DEC). Bayesian analysis recovered *Leishmania* and its subgenera as monophyletic with high bootstrap support values. Both S-DIVA and DEC analyses recovered the Neotropical region as the ancestral area of origin of the *Leishmania* lineage (100%). This result supports the Neotropical hypothesis, were *Leishmania* would have originated 65-40 maa, during the first half on the Cenozoic, and dispersed to the Nearctic and Palearctic regions through land bridges – which coincides with the isolation of the Neotropics (65-55 maa). **Supported by:**CNPq, CAPES, FAPERJ, INCTEM **Keywords:** Biogeography; molecular phylogeny; neotropical hypothesis

PV37 - **IDENTIFICATION OF A NOVEL PENTAMIDINE TARGET IN *T. BRUCEI***

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How could you define life? We, investigators of different life forms, invest a huge amount of energy to understand how organisms live, proliferate and die. But what would be a good criterion for assessing the life of a parasite? Which are the signals telling us that these organisms are alive or not? In our group we evaluate the life of trypanosomatids looking to their nuclear DNA. Cell cycle is an essential process for a successful proliferation finely regulated by specific players and therefore its players can be allies in the fight against highly proliferative cells. Diamidine derivatives, as Pentamdine (Pm), are DNA-binding drugs commonly used against several protozoan diseases. DNA lesions can activate the mechanism of the DNA damage response (DDR) that includes a signal transduction of the sensor molecules to the effectors. The sensors and transducers molecules act throughout the cell cycle, collaborating for the G1/S, intra-S and G2/M cell cycle checkpoints. Two important transducers are ATM and ATR kinases. In this work, we showed by TUNEL assay that Pm generates DNA breaks in *T. brucei* evidenced by DNA fragmentation 2 h after treatment and an increased fragmentation after 12 h. This fragmentation is recovered after 48 h indicating that *T. brucei* has resources to deal with Pm-generated DNA break. We detected gH2A (a DNA damage marker) by immunofluorescence microscopy, indicating an activation of checkpoint pathway after Pm treatment that might be involved with observed DNA repair. To check this possibility, cells were treated with both, ATR and/or ATM inhibitors and Pm and TUNEL remained positive for 48 h. Proliferation growth curves with ATR and/or ATM inhibitors combined with Pm showed significant diminishing of the cell proliferation compared with Pm monotherapy. Taking together, our data showed that a combined therapy with Pm and inhibitors of the cell cycle checkpoint interferes with DNA homeostasis via persistent unrepaired DNA breaks. **Supported by:**FAPESP, CAPES, CNPQ **Keywords:** Dna damage; t. brucei; pentamidine

PV38 - **GENE KNOCKOUT OF A *TRYPANOSOMA CRUZI* RNA BINDING PROTEIN THAT REPROGRAMS EPIMASTIGOTE GENE EXPRESSION AND TRIGGERS METACYCLOGENESIS**

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Trypanosoma cruzi has its genome organized in polycistronic transcription units, implying that post-transcriptional mechanisms have a major role in regulating gene expression. *T. cruzi* RNA binding proteins (RBPs) are key regulators of gene expression since they control maturation, stability, cellular of mRNAs, its translation or cellular localization. Comparative transcriptome analysis of the CL Brener epimastigotes, tissue culture derived trypomastigotes and intracellular amastigotes revealed significant changes in the expression of several RBP genes, including the gene encoding a RBP containing a "zinc finger" motif named TcZC3H99. Transcript levels of TcZC3H99 are 9-fold upregulated in epimastigotes when compared to the other two forms, suggesting a role as a regulator of epimastigote proliferation and differentiation. TcZC3H99 knockout (KO) cell lines generated using CRISPR/Cas9 revealed that epimastigotes mutants grow more slowly when compared to wild-type (WT) parasites. Also, TcZC3H99_KO parasites showed increased metacyclogenesis rates in vitro as well as within the triatomine intestinal tract. RNA-seq analyses comparing TcZC3H99_KO and WT epimastigotes revealed 53 genes with altered transcript levels, including genes encoding amino acid transporters and genes encoding a protein associated with differentiation (PAD), whose mRNA levels were downregulated in TcZC3H99_KO parasites. In contrast, transcripts known to be up-regulated in stationary phase WT epimastigotes, which are undergoing metacyclogenesis, were more abundant in TcZC3H99_KO compared to WT. Co-immunoprecipitation assays of HA tagged TcZC3H99 confirmed the binding capacity of this RBP to transcripts encoding the aminoacid transporter and PAD. Taken together, our data indicate that TcZC3H99 acts as positive regulator of genes related to epimastigote survival and proliferation within the insect vector as well as a negative regulator of genes related to parasite metacyclogenesis. **Supported by:**CNPQ, CAPES, FAPEMIG, INCTV **Keywords:** Rna binding protein; trypanosoma cruzi; rna-seq

PV39 - **A CYTOPLASMIC PROTEIN COMPLEX IS REQUIRED FOR ASSEMBLY OF THE EXTRA-AXONEMAL PARAFLAGELLAR ROD IN TRYPANOSOMES**

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The paraflagellar rod (PFR) is an extra-axonemal structure present in kinetoplastids that runs alongside the flagellum linked to the axoneme and is important for the motility of these parasites. As the PFR has a highly-organised, trilaminar lattice structure, its assembly is likely a regulated and ordered process suggesting the existence of protein partners to help in this process. In a search for potential PFR assembly factors in Trypanosoma brucei, we identified the gene Tb927.10.8870 that encodes a cytoplasmic, coiled-coil protein. We generated Tb8870 null mutants using a CRISPR-Cas9 approach. Immunofluorescence and transmission electron microscopy analysis of the null mutant showed that the PFR is disorganised, much reduced, and the trilaminar organisation was completely absent. In half of the cells, Tb8870 deletion caused an accumulation of unassembled PFR material at the flagellum tip. The disruption to PFR assembly caused a reduced growth rate with errors in cytokinesis. By immunoprecipitation, we identified Tb927.7.1360 as a partner of Tb8870. Tb1360 was also a cytoplasmic, coiled-coil protein and its deletion caused similar defects as seen in Tb8870 null mutants. Our data show that Tb8870 and Tb1360 work together and that important steps in PFR assembly likely occur in the cytoplasm before entry into flagellum, as is found for axonemal components such as the outer dynein arms. **Supported by:**MRC **Keywords:** Paraflagellar rod; flagellum; pfr assembly

PV40 - **ANALYSIS OF THE EFFECTS OF TELOMERASE TERT KNOCKOUT IN *LEISHMANIA MAJOR***

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Leishmaniases are infectious diseases with high mortality and incidence rate caused by parasites of the genus *Leishmania*. The disease is distributed worldwide and presents different clinical manifestations. The lack of efficient treatment protocols and control classify leishmaniasis as neglected diseases urgently demanding an accurate search for new therapeutic targets. Telomerase is a holoenzyme minimally composed by TERT (Reverse Telomerase Transcriptase), and a lncRNA, TER (Telomerase RNA) and its role in telomere length maintenance are crucial for genome stability and cell proliferation. Our work aims to study and better understand the role played by *L. major* telomerase in parasite cell proliferation and survival. We induced knockout (KO) of the gene encoding the parasite TERT component using the CRISPR-Cas9 system developed by Beneke et al. (2017). TERT-double KO parasite clones were isolated using Petri dish selection in M199 supplemented with FBS and the necessary antibiotics (puromycin and G418). The genotypes of the isolated clones were confirmed by PCR, Southern blot, and DNA sequencing. The absence of TERT expression in the isolated KO clones was checked by RT-PCR and Western blot using wild type parasites as a control. Our results show that similar to *Trypanosoma brucei* TERT-KO, in *L. major*, the absence of TERT did not alter cell viability, and parasite proliferation, as well as no visible morphological changes, were detected after few population duplications. The next steps of our work are to verify the impact of telomerase loss in parasite telomere length maintenance, in metacyclogenesis, and parasite infectivity capacity. We also intend to repeat the above experiments using TERT-KO parasites cultivated over a long term and to verify if, in the absence of telomerase, parasites can use alternative means to maintain telomere length to survive. **Supported by:**CNPq; FAPESP **Keywords:** Crispr/cas9; l. major; telomerase

PV41 - **PROTEIN KINASE C INHIBITION MODULATES PROTEOME OF *LEISHMANIA AMAZONENSIS***

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Protein Kinase C (PKC) comprises a group of multifunctional proteins that catalyze phosphorylation in serine and threonine residues. The PKC was characterized in *Leishmania amazonensis* promastigote, related to tegumentar leishmaniasis. During the life cycle, the parasite in the invertebrate host oscillates between procyclical promastigote and metacyclic promastigote. In vertebrate host, the promastigote differentiates into amastigotes inside the infected macrophage. These morphological and metabolic changes are regulated by protein kinases. Due to this, the goal of this work is to investigate the modulation of *L. amazonesis's* proteome by PKC. In this study, we performed a proteomic analysis of promastigote of *L. amazonensis* cultivated with RO32-0432 (PKC inhibitor) for 120h. Combination of FASP method for sample preparation with 'Total Protein Approach' for quantitation of protein abundance allowed the identification of thousands of proteins and the measurement of protein concentrations. Of the 3097 proteins identified, 231 showed changes in their abundance. Analysis of terms enrichment, using gene ontology categories from TriTrypDB revealed that the tRNA aminoacylation for protein translation was the major category downregulated in treated samples (7.61-fold). In addition, a significant decrease in cellular biosynthetic process and cellular amino acid metabolic process, as well in abundance of ribosomal proteins was observed (5.44-, 4.11- and 4.26-fold, respectively). In contrast, treated promastigotes showed an increase in drug metabolic process (8.91-fold), and transmembrane transport (3.77-fold). Furthermore, as a response to the stress caused by the inhibition of PKC, parasites showed an increase of biological regulation of quality (6.20-fold) and oxidation-reduction process (4.18-fold). Taken together, these results demonstrated that inhibition of PKC interfere directly in essential biological processes responsible for *L. amazonensis* homeostasis. **Supported by:**FAPERJ, CNPq, PAPES, CAPES, IOC/Fiocruz **Keywords:** Protein kinase c; leishmania amazonensis; leishmaniasis

PV42 - **DOES DNA REPLICATION CONTRIBUTE TO GENETIC VARIABILITY IN *TRYPANOSOMA CRUZI*?**

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DNA replication is a process that ensures duplication of DNA and inheritance of genetic information, which can be conserved among species or present variability. In the case of *T. cruzi*, high genomic plasticity contributes to the success of the infection by evading the host's immune system. In a search by MFA-seq for origins of replication (ori) of *T. cruzi*, our group identified the presence of ori inside of genes, mainly the ones from the dispersed gene family-1 (DGF-1). DGF-1 has presented a high genetic variability, characterized by punctual mutations. The presence of ori inside of a gene (avoided in other organisms) raised the possibility it would cause replicative stress generated by the collision of the transcription and replication machinery. One hypothesis is that this stress could be the source of the genetic variability of those genes. To validate the locations of the constitutive origins found in the MFA-seq, a ChIP-seq of two proteins belonging to the Origin of Recognition Complex (ORC), Orc1Cdc6 and Orc1b, was performed using Cl Brener cell lineages containing Myc tagged Orc1Cdc6 or Orc1b. The protein-DNA binding intensity profile of the two proteins was generated for triplicates using two *T. cruzi* genomes (Cl Brener and TCC). A fold change between ChIP and input samples was obtained for each replica and we are now identifying all the common peaks between each replica and subtracting them from the control. The heterotrimeric protein RPA is a single-stranded DNA bound protein hence it is present in sites of replicative stress. In order to verify the occurrence of replicative stress inside of DGF-1, a ChIP-seq of HA-tagged RPA-2 was conducted and the data is being processed as described above. After all the final intensity profiles are generated it will be possible to verify if Orc1Cdc6 and Orc1b colocalize with fired origins, if indeed the origins are located inside DGF-1 corroborating the MFA-seq results and if RPA-2 is enriched across DGF-1. **Supported by:**FAPESP **Keywords:** T. cruzi; origins of replication; chip-seq

PV43 - **THE SECRETOME OF THE AVIRULENT PARASITE *TRYPANOSOMA RANGELI***

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Trypanosoma rangeli (Kinetoplastida: Trypanosomatidae) is a non-virulent hemoflagellate parasite of Latin American mammals. Infecting the same hosts and vectors as *Trypanosoma cruzi*, the *T. rangeli* life cycle and survival strategies within its mammalian hosts are not fully understood. Aiming to characterize the *T. rangeli* secretome *in vitro*, epimastigotes from exponential growth phase in LIT medium and *in vitro*-derived trypomastigotes were washed once and cultivated in DMEM pH 7.4 w/o FBS during 6 hours at 27.5°C. After centrifugation, the culture supernatant was submitted to tryptic digestion following mass spectrometry analysis. Preliminary analysis of MS data revealed 122 proteins, among which, 15 were exclusive for epimastigotes and 20 for trypomastigotes. Analysis using Gene Ontology allowed classification of these proteins into 18 different biological functions, among which, “Host-Parasite Interaction” revealed noticeable differences between epi and trypomastigotes as expected. Within this GO category, 41% of the proteins were secreted by both forms but 59% were exclusively secreted by trypomastigotes, including virulence factors as gp63, Complement Regulatory Protein and different trans-sialidases. It is noteworthy to mention that within the “Carbohydrate Metabolism” category no trypomastigote-specific proteins were detected, while 39% of the proteins within this category seems to be exclusively secreted by epimastigotes and might be related to the development of the insect’s stage. Our results indicate a stage-specific secretome for *T. rangeli,* apparently in accordance with the specific and distinct environments faced during the mammalian or the triatomine infection. Such stage-specific secretion of proteins seems also to correlate to the formerly proposed co-evolution of this parasite with its vector species, influencing on the parasite transmission to mammalian hosts. **Supported by:**CNPq, CAPES, FINEP and UFSC **Keywords:** Secreted proteins; developmental stages; host-parasite interaction

PV44 - **CHARACTERIZATION OF DRBD2, AN RNA BINDING PROTEIN WITH TWO RRM DOMAINS AND PARTNER OF POLY (A) BINDING PROTEIN 2 (PABP2) FROM *LEISHMANIA INFANTUM***

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Mature mRNAs are exported from the nucleus as mRNA-protein complexes (mRNPs) and once in the cytoplasm their fates are mediated by RNA-binding proteins (RBPs) that participate in events that include translation, storage and degradation. The RBPs interact with mRNA motifs using functional domains such as the RRM (RNA Recognition Motif). In kinetoplastids, regulation of gene expression relies mainly on post-transcriptional events acting on the mRNAs. However, despite the importance of RBPs in these events there are few characterized studies on their roles. PABP2, a Poly(A)-Binding Protein homologue, has been reported to act during the translation of most mRNAs with a possible additional function during the nucleus/cytoplasm mRNA transport. Previous work found a RBP having two RRMs, DRBD2, co-precipitating specifically with PABP2. Here, we focused on studying DRBD2 further, identifying protein partners and mRNA targets in Leishmania infantum. First, an alignment of several kinetoplastid DRBD2 sequences revealed it to be highly conserved and implying critical functions. Next, cell lines expressing HA-tagged DRBD2 were submitted to immunoprecipitation (IP), with co-precipitated proteins identified by mass spectrometry. As well as PABP2, confirming the previously identified interaction, a large range of proteins was found co-precipitated with DRBD2. These include another PABP (PABP3), a translation initiation factor (EIF4G3), ten other RBPs and nine mitochondrial proteins. To identify mRNA targets also co-precipitated with DRBD2-HA, RNAs were extracted from the IP and used for cDNA synthesis and next generation sequencing. DRBD2 co-precipitated mostly with transcripts coding for hypothetical proteins as well as binding proteins and enzymes, consistent with PABP2-bound mRNAs. These results confirm that the mRNPs formed by DRBD2 are related to the PABP2 function and expand the knowledge regarding their function and putative roles during Leishmania gene expression. **Supported by:**CAPES **Keywords:** Rbp; leishmania; rnaseq

PV45 - **THE *TRYPANOSOMA RANGELI* CYTOKINESIS INITIATION FACTOR 1 (CIF1) ORTHOLOG LACKS CHARACTERISTIC DOMAINS THAT MIGHT INDICATE A DISTINCT FUNCTION**

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Cell division in trypanosomatids starts from the anterior tip of the new flagellar attachment zone (FAZ) towards the posterior end of the cell. In *Trypanosoma brucei*, CIF1 is also called as “Tip Of Extending FAZ” (TOEFAZ1), being essential for targeting cytokinesis regulators to their functional site. Due conflicting data on the *Trypanosoma rangeli* ability to perform division within mammals and the lack of description of CIF1 for this taxon, we aimed to investigate the presence and the expression of this protein. Comparative genomics allowed identification of CIF1 ortholog in the *T. rangeli* genome, 29.72% identical to the *Tb*CIF1 and maintaining a conserved synteny, but lacking the coiled-coil and the zinc-finger domains required for its activity, indicating a possible loss of function as also observed for *Trypanosoma cruzi* and *Leishmania* sp.. Further investigation by qPCR confirmed the transcription of this gene, revealing mRNA levels 2.72 times higher in trypomastigotes when compared to epimastigotes. Heterologous expression of a ~12kDa fragment corresponding to the C-terminus allowed generation of a polyclonal antiserum in mice. Western Blot assays using this antiserum recognized a protein of ~69 kDa in epimastigote and trypomastigote extracts, indicating the expression of this putative *Tr*CIF1 ortholog, but also an additional weak recognition of a protein of ~160 kDa. Preliminary IFA indicate a diffuse cytoplasmic distribution of the protein in epimastigotes but a stronger flagellar localization in trypomastigotes, being distinct than previously reported for *Tb*CIF1. Surprisingly, a clear and consistent protein concentration was observed in the flagellum tip of the non-dividing *T. rangeli* trypomastigotes. So far, our results seem to indicate that this putative CIF1 ortholog in *T. rangeli* might not be related to the parasite cytokinesis as reported for *T. brucei*, suggesting a possible different function. **Supported by:**UFSC, CNPq, CAPES and FINEP **Keywords:** Toefaz1; cell division; flagellum

PV46 - **THE GP63 METALLOPROTEASE FAMILY OF *TRYPANOSOMA RANGELI***

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Trypanosoma rangeli is a parasite that shares different host species with *Trypanosoma cruzi*, however it is considered nonpathogenic to mammals. These parasites also share genes and proteins, among them, GP63 is a metalloprotease found mainly in the parasites surface and it is involved in the host-parasite interaction. In pathogenic parasites, as *T. cruzi*, *Trypanosoma brucei* and *Leishmania*, GP63 is an essential virulence factor. Its enzymatic function is dependent of a zinc bond with three histidine residues and a water molecule bonded to a glutamic acid present in the motif (HExxHxxGxnHx+M). The *T. rangeli* GP63 is a protein family classified into 3 groups, related to the amino acid composition differences in the catalytic site. In order to better understand those differences, this study aimed to characterize the GP63 groups in *T. rangeli*. A search was performed for GP63 members on the *T. rangeli* genome, finding 131 complete sequences: 109 were classified as GP63_1 (HExxH), 18 as GP63_2 (HAxxH) and 4 as GP63_3 (RGLAY). In phylogenetic analysis, all GP63_2 sequences were grouped in the same clade, as well as the GP63_3 members, being both groups originated by different GP63_1 members. GP63 mRNA sequences were found in the transcriptome of *T. rangeli*, and at least 8 genes were differentially transcribed among the biological forms. GP63 proteins were identified on the secretome of *T. rangeli* epimastigotes and trypomastigotes, however GP63_1 was secreted only by trypomastigotes, while GP63_2/3 were secreted by both forms. Tridimensional structures were performed for one member of each GP63 group and it was observed that the differences on the motif can affect the catalytic activity of these enzymes, since the motif of the GP63_2/3 may not coordinate the zinc. These results suggest that, even though GP63_2/3 proteins probably do not exert metaloprotease activity, they are still being transcribed and expressed, possibly revealing a distinct function in *T. rangeli*. **Supported by:**UFSC, CNPq, CAPES and FINEP **Keywords:** Structural modeling; transcriptome; metzincins

PV47 - **CHANGES IN GLUCOSE METABOLISM OF *TRYPANOSOMA CRUZI* EPIMASTIGOTES INDUCED BY HEME**

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Chagas disease is a neglected illness caused by *Trypanosoma cruzi*. Epimastigote forms inhabit triatomine insects midgut, an environment rich in heme, a molecule that has been shown to increase proliferation. Transcriptome of heme-treated epimastigotes evidenced an increase in the expression of genes related to glycolytic metabolism, suggesting that fermentative pathways may be responsible for energy homeostasis. Thus, the aim of the study was to evaluate the glucose (glc) metabolism of heme-treated epimastigote. After seven day of culture, parasites treated with heme+glc presented a greater proliferation compared to control. Interestingly, after ten days, parasites cultured with heme+glc showed a significant increase in proliferation when compared to parasites that were cultured only with heme. We also observed that, from the 7th day of culture, heme-treated parasites decreased in 55% glc from medium even without glc supplementation. Moreover, parasites that were treated with heme+ glc reduced 84% glc in the medium compared to glucose supplemented parasites. The increment in glc consumption was also accompanied by medium acidification. Additionally, the inhibition of glycolysis with 2-DG reduced 55% of heme-induced proliferation, an effect not observed when parasites were treated with 2-DG alone. The respirometric results showed that basal oxygen consumption of intact heme-treated parasites presented an increase, compared to control group after five and seven days of culture. Glc addition reduced parasites respiration only in the presence of heme, followed by a decrease in ETS. However, extramitochondrial respiration was not significantly different between heme-treated and control parasites. In summary, our results suggest that *T. cruzi* epimastigotes seem to, in the presence of heme, prefer to consume glucose as a main carbon and shift glucose catabolism into fermentation decreasing respiration, in order to sustain parasites proliferation. **Supported by:**FAPERJ, CNPq and INCT-EM **Keywords:** Trypanosoma cruzi; heme ; energy metabolism

PV48 - ***TRYPANOSOMA CRUZI* RNA-BINDING PROTEIN DRBD3: PERINUCLEAR FOCI FORMATION DURING BENZNIDAZOLE EXPOSURE.**

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Chagas disease is one of the most harmful neglected tropical disease in the Americas. Currently, benznidazole (BZ) is the trypanocidal compound of choice for Chagas disease treatment. However, this drug often fails to cure the infection. The regulation of gene expression in *Trypanosoma cruzi*, the causative agent of Chagas disease, is based on post-transcriptional mechanisms. When sudden environmental changes cause translational arrest, RNA-binding proteins, and their target mRNAs assemble into cytoplasmic bodies, known as RNA granules, which act as RNA sorting centers. We have characterized the *T. cruzi* RNA-binding protein DRBD3, which is conserved among trypanosomatids. TcDRBD3 structural analysis showed that this protein has two RRMs domains, predicted as members of the polypyrimidine tract-binding protein subfamily, and has a C-terminal low-complexity sequence rich in proline and glutamines. Using a recombinant form of TcDRBD3 to facilitate its detection (rTcDRBD3), we showed that this protein resides in the cytoplasm, but localizes into perinuclear cytoplasmic foci after BZ exposure. RNA staining after BZ also showed that this molecule accumulates into perinuclear cytoplasmic foci. Moreover, BZ and puromycin treatment enhanced the colocalization of rTcDRBD3 and RNA, suggesting that TcDRBD3 granules repertoire also harbors RNAs released from polysomes. Under starvation, rTcDRBD3 granules localized throughout the cytoplasm and also increased in number in the presence of puromycin. Our results suggest that, after BZ exposure, TcDRBD3 accumulates into perinuclear granules that harbor RNA and also that its localization varies according to the type of stress. **Supported by:**CAPES, CNPq, FAPEMIG **Keywords:** Rna binding proteins; benznidazole stress; perinuclear granules

PV49 - **FUNCTIONAL CHARACTERIZATION OF *TRYPANOSOMA CRUZI* GIANT PROTEIN USING CRISPR / CAS9 SYSTEM**

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In the Trypanosomatidae family, we demonstrated the presence of giant proteins at the Flagellar Adhesion Zone (FAZ). Recently, our group characterized the FAZ10 giant protein in Trypanosoma brucei, at the FAZ intracellular domain. Ablation of FAZ10 impaired the timing and placement of the cleavage furrow during cytokinesis, resulting in premature or asymmetrical cell division. However, the function of the cytoskeletal giant protein in T. cruzi remains unknown. This project aims to investigate the role of T. cruzi giant protein using the CRISPR/Cas9 system and identify the cytoskeletal elements that interact with this protein at different stages of the parasite life cycle. A mass spectrometry analysis of T. cruzi proteins (≥500 kDa) was excised from an SDS-PAGE gel, resulting in peptides data matched against the TriTrypDB. Calpain-like cysteine peptidase - CLP (XP_809993) with three calpain domains was identified with 18 sequence hits, encoded by a 13,716bp, predicted to be a giant protein (≥519 kDa) and the isoelectric point is 5.22. Based on this information, we generate a synthetic peptide (18 aa) to the first calpain domain coupled to the epoxy-activated sepharose resin. The synthetic peptide was used for specific antibody production by mice immunizations. A CLP fragment was fused to the glutathione-S-transferase (GST) coding sequence in the pGEX-4T-1 vector to generate a recombinant protein for protein-protein interaction assays. To study the CLP function, we designed single-guide RNAs (sgRNAs) and DNA donor-specific to specific calpain domains. T. cruzi CL Brener epimastigote forms containing Cas9: GFP integrated into the genome, will be transfected with sgRNAs and DNA donor followed selection with G418. The characterization of its biological function may provide answers about this organism and, in the future, contribute to developing new drugs to control Chagas' disease. **Supported by:**CNPq **Keywords:** Faz; trypanosoma cruzi; crispr/cas9 system

PV50 - **IDENTIFICATION OF DIFFERENT MRNA POPULATIONS ASSOCIATED TO TWO POLY-A BINDING PROTEINS HOMOLOGUES (PABP2 AND PABP3) IN LEISHMANIA INFANTUM**

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PABPs, the poly-A binding proteins, bind specifically to the poly-A tail found on the 3' end of eukaryotic mRNAs and are known to have major roles associated with all aspects of their metabolism, including translation and regulation of half-life. Three PABPs are found in Leishmania with roles that still need to be better defined. Previous studies have shown that PABP1 interacts with ribosomal protein mRNAs, while PABPs 2 and 3 appear to be associated with other mRNA populations. This work aimed to define the mRNAs bound by PABPs 2 and 3. First, L. infantum cells were transfected for the expression of HA-tagged version of the three PABPs, followed by generation of cytoplasmic extracts where the expression of each PABP was confirmed by Western blots using a commercial anti-HA antibody. PABPs immunoprecipitations were then performed, followed by subsequent extraction of the co-precipitated RNAs, cDNA synthesis and large scale sequencing. Based on the sequenced data, a differential expression analysis of the bound mRNAs confirmed a lack of overlap in mRNA content bound by PABP1xPABP2 or by PABP1xPABP3. In contrast, no differences in mRNAs associated with PABP2 or PABP3 can be detected. Among the mRNAs associated with PABP2 and PABP3, only 3 to 4% were classified as coding for ribosomal proteins. For both proteins ~40% of the transcripts encode proteins with catalytic activity and another ~40% with binding function, including histone mRNAs, while ~10% of the genes encode proteins with transporter activity. When compared with previous experiments carried out with native PABPs, using antibodies with some cross-reactivity to different PABP homologues, the experiments with the HA-tagged proteins were much clearer with PABP1 having a much stronger preference for mRNAs encoding ribosomal proteins. This analysis confirms major distinctions in mRNA targets bound by PABP1 or PABP2/PABP3 that are possibly associated with defined fates or regulatory events directed to these mRNAs. **Keywords:** Translation; pabp; rnaseq

PV51 - **MOLECULAR CHARACTERIZATION OF THE PLASMODIUM VIVAX HSP70 PROMOTER SEQUENCE USING HETEROLOGOUS TRANSFECTION**

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Plasmodium parasites have a complex life cycle involving transmission between arthropod and vertebrate hosts. Changes in the microenvironment between different tissues and hosts require thin regulation of gene expression. Expression control relies on transcriptional and post-transcriptional mechanisms. Transcriptional control can be achieved by regulation of chromatin conformation, enabling or disabling access of DNA sequences to the RNA polymerase complex; or regulation of RNA polymerase activity by Transcription Factors (TFs), proteins that bind DNA sequences. Comparative genomics and proteomics analysis detected few TFs in Plasmodium, suggesting the presence of TFs unrelated to previously described DNA domains. New molecular studies are necessary to identify functional new TFs and domains in Plasmodium. Furthermore, Plasmodium transcriptional control studies are restricted to P. falciparum, species evolutionary distant from other species that cause human malaria, such as P. vivax, the main cause of malaria in Brazil. The lack of long term in vitro culture of P. vivax is a challenge for malaria research. Due to genetic differences, P. vivax promoters are not recognized by P. falciparum transcriptional machinery. Advances of in vitro cultivation of P. knowlesi, a species close to P. vivax, provide a new model. Recently, we observed that P. vivax promoter sequences are recognized by P. knowlesi machinery, indicating that this model can be used for regulatory sequence studies. In this project, we aim to characterize P. vivax promoters using transfection of P. knowlesi with plasmids containing different P. vivax promoters driving luciferase expression. Results show that tha *P.vivax hsp 70* gene promoter is fuctional in *P.knowlesi* and *P.faciparum,* containing sequences domains recognized by the machinery of both species. The identification os these sequences will help to elucidate mechanisms of transcriptional control in malaria parasites. Suported by FAPESP,CNPq. **Supported by:**Fapesp **Keywords:** Malaria; transcription; transfection

PV52 - **IDENTIFICATION OF SEQUENCE MOTIFS COMMON TO MRNAS TARGETED BY THE RNA BINDING PROTEIN RBP23 FROM LEISHMANIA INFANTUM AND TRYPANOSOMA BRUCEI.**

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Translation control of gene expression in trypanosomatids is likely to be related to events involving the poly-A tail binding protein (PABP), bound to the 3' end of the mRNAs, as well as other RNA binding proteins (RBPs) having distinct RNA binding domains. Three PABPs are found in these organisms, with two of them directly implicated in translation (PABP1 / 2). RBP23 is a RBP that specifically co-precipitates with PABP1 and the translation initiation factor EIF4E4, a known PABP1 partner. All three proteins were found to specifically associate with mRNAs encoding ribosomal proteins (RP-mRNAs), with RBP23 having a nearly exclusive preference for these mRNAs. RBP23 has thus been proposed to mediate mRNA recognition by its protein partners. Based on previous chip-seq data, that enabled the identification of the mRNAs most strongly associated with L. infantum RBP23, this work aimed to investigate the RNA-binding preferences of RBP23. Our goal was to identify sequence elements that may contribute to the specific RBP23 association with RP-mRNAs in T. brucei and Leishmania. Here, these transcripts were all predicted to have short 3' UTRs (~300 nts in length) and minimal 5' UTRs (<50 nts in general, but with some messages having 5'UTRs <10 nts in length). When both sets of UTRs were submitted to MEME analysis for identification of common sequence motifs, only motifs within the 3'UTRs were found and indeed the absence of motifs within the 5'UTRs was expected due to their small sizes. Within the 3'UTRs, 15 bp motifs enriched with thymine were found several times in most RP-mRNAs from both L. infantum and T. brucei, a finding that was statistically significant. A unique transcript lacking these motifs was found bound to RBP23 and this was its own mRNA, presumably involved in processes that are unrelated to those linked to the RP-mRNAs. The identified motifs might then be true RBP23 binding targets, a possibility that yet needs to be proven experimentally using chimeric mRNAs. **Supported by: Keywords:** Translation; rbp; pabp

PV53 - **IDENTIFICATION AND CHARACTERIZATION OF AN OXIDOREDUCTASE IN *LEISHMANIA AMAZONENSIS.***

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Leishmania is a protozoan parasite that causes leishmaniases, diseases with clinical manifestations ranging from cutaneous lesions to visceral damage. *Leishmania* is auxotrophic for L-arginine and presents the machinery for its uptake and metabolism. The amino acid is the common substrate for the enzymes arginase, involved in the polyamines production, and nitric oxide synthase (NOS), involved in nitric oxide (NO) production. Arginase activity is essential for *Leishmania amazonensis* (La-WT) survival, as well as NO production for promastigote metacyclogenesis and amastigote differentiation. Also, the knockout of arginase in *L. amazonensis* (La-arg-) altered the production of NO in promastigotes and amastigotes forms.
The aim of this project is to identify and characterize the gene encoding an oxidoreductase protein which has a *L. mexicana* homolog, *Lmx*M19.1450, oxidoreductase-like protein - GI 401419669 -, location 2973-4725, KEGG 1.14.13.39, that possibly is the NOS-like enzyme responsible for NO production during promastigote growth and amastigote differentiation. To do that, the nucleotide sequence of the ORF of the gene was amplified using *L.amazonensis* genomic DNA as the template and the PCR product was ligated into an overexpression plasmid (pSNBR) and transfected. The transcription of the gene in transfected *L.amazonensis* was analysed using RT-qPCR.
We were able to identify the gene in *L.amazonensis* and to compare its similarity with other *Leishmania* species, obtaining a 99% identity for *L. mexicana*, 94% identity for *L. infantum* and 93% identity for *L. major*. The gene was also overexpressed in *La*-WT using the pSNBR vector. The overexpression of the NOS-like could allow new insights into the NO production and parasite infectivity. We were also able to analyze the NOS-like gene mRNA expression in different stages of the growth curve and infection in BALB/c, C57BL6 and THP-1 derived macrophages. **Supported by:**Fapesp **Keywords:** Leishmania; nitric oxide; arginase

PV54 - **IDENTIFICATION OF PROTEIN PARTNERS AND INTERACTIONS INVOLVING THE CAP BINDING PROTEIN EIF4E6 IN *TRYPANOSOMA BRUCEI***

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Gene expression in trypanosomatids is regulated mainly by post-transcriptional mechanisms and translation initiation may be an important target for such mechanisms. In eukaryotes, translation initiates with the recognition of the mRNAs by the eIF4F complex, formed by three subunits: eIF4A, eIF4G and the cap binding protein eIF4E. In trypanosomatids, six eIF4E and five eIF4G homologues have been described, forming multiple eIF4F-like complexes with likely roles in regulatory events. The complex EIF4E6/EIF4G5/EIF4G5-IP was originally described in *Trypanosoma brucei* procyclic cells, but it is not well known. Here we first aimed to further characterize this complex in *T. brucei* bloodstream forms (BSF), in order to identify/confirm functional partners. The proteins of interest were expressed in BSF as C-terminally PTP-tagged polypeptides and their expression confirmed through western blot using anti-Protein A antibody. These were followed by co-immunoprecipitation (CoIP) assays. Mass spectrometry analysis of the CoIPs confirmed that the EIF4E6/EIF4G5/EIF4G5-IP complex is present in BSF and identified several proteins co-precipitating with it, several involved in flagella formation and mRNA metabolism. Next, we investigated direct interactions between selected partners. Through *pull-down in vitro* assays, EIF4E6 was seen to interact directly with both EIF4G5 and EIF4G5-IP. To map relevant motifs involved in the binding between these proteins, mutations were tested in two conserved EIF4E6 residues likely involved in protein-protein interactions (W43A and H51A). Neither, however, was able to abolish EIF4E6 binding to EIF4G5 or EIF4G5-IP. Regarding the EIF4E6/EIF4G5 interaction, it was mapped to the EIF4G5 N-terminal region, since mutations in two amino acid residues (EF91-92AA) abolished its binding to EIF4E6. These results contribute to a better understanding of the events leading to formation of this complex in trypanosomatids, with possible functional implications. **Supported by:**CNPq, FACEPE, FIOCRUZ **Keywords:** Eif4f; protein interaction; bloodstream form

PV55 - **FUNCTIONAL CHARACTERIZATION OF ZC3H41, A ZINC FINGER CCHC-TYPE PROTEIN OF *LEISHMANIA INFANTUM* THAT CO-PRECIPITATES WITH POLY(A) BINDING PROTEIN (PABP) HOMOLOGUES**

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Initiation of eukaryotic protein synthesis is dependent on the recognition of the mRNA 5' end by the heterotrimeric translation initiation complex eIF4F, in association with the Poly-A Binding Protein (PABP) bound to the mRNA's 3' end poly-A tail. Regulation of translation of specific mRNAs is in many cases dependent on their recognition by RNA Binding Proteins (RBPs) that might enhance/prevent aspects of the eIF4F/PABP functions. In Leishmania species, three PABP homologues are present that bind to different eIF4F complexes, RBPs and mRNA targets, but whose functional distinctions need to be better defined. Previous studies showed that the ZC3H41, a Zinc Finger CCHC-type protein, co-precipitates with PABP1 in Leishmania infantum, indicating that this RBP might have a role during translation in association with PABP1. This study aimed to characterize L. infantum ZC3H41 in order to provide insights into the PABP1 function. L. infantum cells expressing HA-tagged ZC3H41 were first generated, with the ectopic protein showing a constant expression in promastigotes and no indication of post-translational modifications. Cytoplasmic extracts of these cells were used for immunoprecipitation (IP) and subsequent mass spectrometry, which confirmed the ZC3H41 co-precipitation not only with PABP1 but also with PABP2 and PABP3. ZC3H41 also co-precipitated with other PABP1 partners: RBP23, LinJ.18.0300, LinJ.05.0450. Pull-down assay with all three GST-tagged PABP and radiolabeled ZC3H41 corroborated the in vivo results showing a direct interaction between ZC3H41 and all three PABP homologues. New IPs were performed to identify mRNAs bound to ZC3H41 through RNAseq and these revealed that over 70% of the transcripts bound to ZC3H41 encode ribosomal proteins, similar to those bound to PABP1. These results implicate ZC3H41 as a component of distinct mRNA binding complexes associated with different PABPs and protein partners, but with yet undefined roles in mRNA translation/metabolism. **Supported by:**FACEPE **Keywords:** Zinc finger; leishmania; pabp

PV56 - **EXPLORING THE HISTONE H2B VARIANT FUNCTION AND ITS INVOLVEMENT IN TRANSCRIPTION START SITES**

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Trypanosoma cruzi, the etiological agent of neglect Chagas disease, constantly transits from different microenvironments to proceed its life cycle. Nuclear and chromatin structure changes happen simultaneously during the cell cycle and life cycle. Recently, epigenetics studies of histones post translation modifications (PTMs) and histones variants depositon suggest a putative association to genic expression regulation among its life forms. We previously showed that histone H2B variant (H2BV) is enriched in *T. cruzi* trypomastigote forms, and therefore, we are looking for its role in chromatin regulation. H2BV Heterozygotes knockouts parasites differentiate more into metacyclics than wild type, and are more abundant at S phase of cell cycle. By H2BV pulldown assays, we detected that H2BV associated (among others) to the protein bromodomain factor 2 (BDF2), which is known to interact with acetylation residues in histones, exclusively in trypomastigote forms. Currently, we performed a reverse pulldown using recombinant BDF2. Mass spectrometry analysis of the eluates indicates interaction with histone H4, H2B and H3, corroborating previous observation, evincing its association with *T. cruzi* nucleosomes. Moreover, we are currently analysing H2BV ChIP-seq data from epimastigotes, trypomastigotes and amastigotes for determination of its genomic localization, possibly confirming the deposition in transcription start sites, as observed in *T. brucei.* **Supported by:**CAPES, FAPESP **Keywords:** Histones; transcription start sites; chip-seq

PV57 - **USE OF GENE EDITING PROTOCOLS USING CRISPR/CAS9 TECHNOLOGY IN *LEISHMANIA INFANTUM***

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CRISPR/Cas9 technology has been shown to be a powerful tool ... or knockin. In *Leishmania*, there are studies using CRISP... other advances, the possibility to validate new targ... breakdown of the double DNA strand (DSB) ge... mediated end joining (MMEJ) or by ho... involvement of these mechanisms for ... we tested protocols using epi... expression of Cas9 of *Stre*... the *L. infantum* miltef... with two differe... with a dono... flanke... in t... Sequ... prese... gene se... codon. T... constitutiv... MMEJ in *Leishmania*. In addition, they indicate that the CRISPR/Cas9 platform has been successfully implemented, allowing its use in future studies of gene function and development of vaccines and new therapeutic targets in *Leishmania*. **Supported by:**CNPQ; CAPES; INCTV **Keywords:** Crispr/cas9; leishmania ; miltefosine transporter

PV58 - **EFFECTS OF THE MILTEFOSINE TRANSPORTER KNOCKOUT THROUGH CRISPR/CAS9 GENE EDITING ON LEISHMANIA AMAZONENSIS VIRULENCE**

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In the last few years, the CRISPR/Cas9 system has been proven as an important tool in studies of gene function. The knockouts and knock ins induced by this system allow better understanding of cellular, metabolic or structural impacts caused by addition or removal of some component, improving understanding of several aspects that may further lead to vaccine, diagnostic or therapeutic interventions. CRISPR/Cas9 has already been applied in different studies for different leishmania species. Our research group has an undergoing study with *Leishmania infantum* and *Leishmania amazonensis*, two of the most important etiologic agents of visceral and tegumentary leishmaniasis. In the present work, we tested different CRISPR/Cas9 protocols to introduce knockout on *L. amazonensis* Miltefosine Transporter as a proof of concept. We've induced Cas9 expression by transfection with two plasmids (episomal and integrative). We expressed recombinant *Staphylococcus aureus* Cas9 (SaCas9) and assorted its activity in vitro and by transfecting promastigotes. We still aimed to evaluate the reported association between miltefosine resistance and virulence of Leishmania. To do so, knockout miltefosine resistant parasites will be used for *in vitro* (bone marrow derivated macrophages) infection and *in vivo* assays utilizing BALB/c model (wound progression and parasitic burden evaluation). We hope to improve protocols for the use of CRISPR/Cas9 in *L. amazonensis* and to contribute to better understanding of the mechanisms associated with virulence in miltefosine resistant parasites. **Supported by:**FAPEMIG **Keywords:** Crispr/cas9; leishmania; miltefosine transporter

PV59 - **INTERACTION MECHANISMS BETWEEN EUKARYOTIC INITIATION FACTORS AND POLY-A BINDING PROTEINS IN *TRYPANOSOMA CRUZI***

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During translation initiation, canonical circularization of a eukaryotic mRNA is mediated by eIF4G and PABP. However, using the two-yeast hybrid assay we demonstrated the interaction of two *Trypanosoma cruzi* PABP (TcPABP1 and TcPABP2) with the eIF4E homologue 3 (TcEIF4E3) but not with the eIF4G. A similar interaction was described for *Leishmania infantum* EIF4E homologue 4 (LiEIF4E4) which shares with eIF4E3 an amino-terminal extension with three PAM2 motifs (“A”, “B” and “C”). The interaction between LiEIF4E4 and LiPABP1 maps in PABP1 MLLE domain with at least PAM2 “B” in eIF4E4. Since a direct eIF4E:PABP interaction has not been described in mammals, this important difference on translation machineries of trypanosomatids and mammalian cells makes a promising target for the development of anti-parasitic drugs. Our aim is to validate these interactions in *Trypanosoma cruzi* and further study the molecular mechanisms involved. We are currently working on the immunoprecipitation of the complexes formed *in vivo* by these proteins using polyclonal antibodies in wild type parasites or anti-GFP antibodies in GFP-tagged transfectants. In addition, we are mapping the regions mediating the interactions using recombinant truncated constructs. The TcEIF4E3 constructs contain either the entire amino-terminal extension (with the three PAM2 “ABC”) or the “AB” or the “BC” regions in fusion with GFP. The MLLE domain of the two TcPABP homologues are expressed as thireodoxin fusions and cleaved with TEV protease. Although the TcEIF4E3 amino-terminal region is intrinsically disordered, we successfully purified the TcEIF4E3 N-terminal GFP-fused constructs. The binding affinity between the recombinant proteins will be determined by microscale thermophoresis. This, together with the validation of the interactions *in vivo* should contribute to the understanding of this trypanosomatid-specific mechanism of translation regulation and possible novel mechanisms of gene expression regulation. **Supported by: Keywords:** Trypanosoma cruzi; eukaryotic initiation factor (eif4e); poly-a binding protein (pabp)

PV60 - **REVISITING THE UNIVERSE OF NON-CODING RNAS IN *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi is a protozoan parasite responsible of Chagas disease, a neglected parasitic disease. *T. cruzi* genome assemblies remained highly fragmented as a consequence of the intrinsic genome complexity (abundance of repetitive sequences and genes organized in tandem), up until the recent advent of long-read sequencing technologies (PacBio and Oxford Nanopore), which improve genome sequencing contiguity. Even though several efforts were made in order to correctly annotate coding genes and repetitive sequences, non-coding RNA annotation has not been thoroughly assessed. The peculiar mechanisms of RNA metabolism in *T. cruzi* and the improved genomes’ assemblies prompted us to study how the non-coding RNA genes are organized in the genome. We used several optimized algorithms depending on the RNA to re-annotate them providing a complete annotation, including the identification of previously undescribed non-coding RNAs as well as the correct annotation of genes that were previously incorrectly assigned. In sum, this work reports a highly curated genome annotation, and unveils the organization of non-coding RNAs in the genome assembly of *T. cruzi*. **Supported by: Keywords:** Non-coding rna; annotation; genome organization

PV61 - **FISH ANALYSIS REVEALS INTRA-STRAIN HETEROGENEITY OF PLOIDY AND CHROMOSOMAL MOSAICISM IN TRYPANOSOMA CRUZI**

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Aneuploidy is an important factor in generating genetic variability in fungi and protozoan parasites. Studies using whole genome approaches (NGS, CGH, karyotyping) indicated the presence of aneuploidy in T. cruzi, but the degree of aneuploidy is not yet known. FISH is a powerful tool for evaluation of ploidy at single cell level, and whether it occurs across all cells or in a fraction of cell population. In this work we investigate the presence of aneuploidy in T. cruzi by FISH. We used chromosome-specific markers that are single or low copy number genes. Due to the small size of T. cruzi nucleus, the protocol has been adapted for detection of low copy number genes. For the analysis we chose the gene H49 located on the chromosome TcChr39 of CL Brener. The central region of H49 is composed of 204-bp tandem arranged repeats in an uninterrupted large array producing a good target for hybridization.
H49 repeats were labeled with Digoxigenin-11-dUTP, cleaved with Dnase I into ≤ 100 bp fragments and incubated with permeabilized epimastigotes. Signals were revealed with anti-Digoxigenin and Alexa 488. Nuclei and kinetoplast were labeled with DAPI. Using an anti-flagellin antibody we estimated the presence of 5% mitotic cells in the sample and they were excluded from the analysis. Total number of labelled TcChr39 chromosomes were estimated by epifluorescence microscopy by two independent observers. No statistically significant differences were found between the counts. Ploidy was estimated by counting approximately 500 cells. The efficiency of chromosome labelling by FISH was 80%. Data showed that 26.2% of cells were monosomic, 36.1% disomic and 18.2% trisomic. Only 3.1% of population had polyploid events which were not considered in the analysis. Aneuploidy events were confirmed by confocal microscopy. TcChr39 chromosome was observed in three ploidy states, producing an intra-strain heterogeneity of ploidy, suggesting the occurence of chromosomal mosaicism in T. cruzi. **Supported by:**FAPESP, CNPq, CAPES **Keywords:** Trypanosoma cruzi; fish; aneuploidy

PV62 - **RIBOSOMAL PROTEINS IN *LEISHMANIA MAJOR***

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Ribosomal proteins (RPs) are considered a burden in proteomic analyses because of their abundance in the cell. Nevertheless, studies reporting non-canonical activities of these proteins, apart from the ribosomal composition, have shown that several of these proteins, in different organisms, play a regulatory role in multiple cellular processes. In this context, the objective of this work is to study ribosomal proteins in the Leishmania parasite. We chose two of them, the RPS16 and RPL19, which are transcribed from duplicated genes in Leishmania major. Computational analysis showed that RP S16 transcripts have divergent UnTranslated Regions (UTRs) and identical protein coding sequence (CDSs), and the RP L19 genes have divergent UTRs, in addition to non-identical CDSs. Divergent UTRs may be associated with differential expression control and divergent CDSs to loss or gain of function. To study these proteins and corresponding transcripts we are evaluating their expression profile and subcellular localization. Genome editing of Leishmania major, using the CRISPR/Cas9 system, was conduct to produce knockout transfectants for each copy of the S16 and L19 genes and to myc tag each one of the encoded proteins. Transfectants containing the two copies of myc-labeled RP S16 were obtained and protein levels were analyzed by Western Blotting with commercial anti-myc antibody. Transfectants with the planned knockouts for each copy of RP S16 are under analysis, as are the myc tagged transfectants of the RP L19 copies. The present study opens the opportunity to investigate unconventional roles of ribosomal proteins. **Supported by:**FAPESP e CAPES **Keywords:** Leishmania; ribosomal protein; extraribosomal function

PV63 - **A NOVEL MEMBER OF THE PROGESTIN AND ADIPONECTIN FAMILY OF RECEPTORS (PAQRS) THAT BINDS TO PLATELET-ACTIVATING FACTOR (PAF) AND LYSOPHOSPHATIDYLCHOLINE (LPC) IN THE PARASITE *TRYPANOSOMA CRUZI***

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Lipid mediators, including lysophosphatidylcholine (LPC) and platelet activation factor (PAF), present relevant roles during *T. cruzi* infection. Studies on processes stimulated by phospholipids, such as LPC and PAF, are important for the understanding of essential mechanisms for the maintenance of the *T. cruzi* life cycle, such as cell differentiation and host cell invasion. Previous studies from our group suggest the existence of a PAF receptor in *T. cruzi* (TcPAFR), involved in parasite differentiation and host cell infection by the parasite. In the present work, we obtained a mutant phenotype for the TcPAFR gene, our goal was to identify and characterize TcPAFR through bioinformatics tools. In parallel, we proposed a model for the protein and for the interaction of this receptor with PAF, LPC and WEB 2086 (PAF antagonist receptor) through molecular modeling and docking. We also evaluated whether mutant parasites for the PAFR (KD) would respond differently from the wild-type (WT) parasites to the stimuli caused by these phospholipids in their metacyclogenesis. The TcPAFR has been identified as homologous to a family of 7 transmembrane domain receptors that bind to adiponectin and progesterone in mammals (PAQR). We observed that all ligands were positioned in the cavity formed by the seven transmembrane helices. Lysine 212 and alanine 213 residues were able to interact with the 3 ligands analyzed. When parasites were treated with LPC or PAF, the percentage of metacyclic trypomastigotes in WT cells was significantly higher than in the KD group and the untreated flagellates, being up to 20% more metacyclic trypomastigotes. The increase in differentiation of WT parasites, stimulated by LPC and PAF, was abrogated in the presence of WEB 2086. Our data indicate that LPC and PAF probably act through this receptor in *T. cruzi*. Undergoing experiments aim to improve the knowledge of these mechanisms, which may get to developing chemotherapeutic agents against these parasites. **Supported by:**CAPES, CNPQ, INCTEM **Keywords:** Lipids; modeling; metacyclogenesis

PV64 - **EDITING OF DELTA-AMASTINS OF TWO *TRYPANOSOMA CRUZI* STRAINS**

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Amastins are small surface glycoproteins initially described as differentially expressed genes upregulated in amastigote stages of *Trypanosoma cruzi*. However, *in silico* analysis have shown that amastins are present in all Trypanosomatids, and can be classified as α, β, γ and δ-amastins. Meanwhile, the δ-amastins, which were the first described, can be stratified in δ-amastin and δ-Ama40/50. Delta-amastins are up-regulated in initial steps of intracellular stage, this aspect suggests that these proteins could fulfill important functions in the host-parasite interaction, as well as the parasite's survival and virulence capacity. This work aimed to improve the functional characterization of δ-amastins in the CL Brener and Dm28c clones, by editing through the use of the CRISPR-Cas9 system. For this, a sgRNA, that can target all delta-amastin copies from several *T. cruzi* strains, was designed and *in vitro* transcribed. In addition, an oligonucleotide donor sequence containing three stop codons plus a *Bam*HI site to disrupt the coding sequence were designed and co-transfected with complex of *Sa*Cas9 plus sgRNA in Dm28c and CL Brener clones. Gene editing in both populations and clones were confirmed by RFLP-PCR, and the parasites are currently being tested for phenotypic alterations during infection. **Supported by:**CAPES and CNPq **Keywords:** Delta-amastin; cl brener, dm28c ; crispr-cas9 system

PV65 - **CLONING AND EXPRESSION OF *TRYPANOSOMA CRUZI* GIANT PROTEIN FRAGMENT TO PERFORM INTERACTION WITH CYTOSKELETAL PROTEINS**

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Chagas' disease is caused by *Trypanosoma cruzi* and affects 8 million people, mostly in Latin America. In trypanosomatids, including *T. cruzi*, the presence of a giant protein (PGs) family with high molecular mass (≥ 500 kDa) has been demonstrated. A mass spectrometry (MS) analysis of a fragment excised from an SDS-PAGE identified a calpain-like cysteine protease (CLP) in the *T. cruzi* cytoskeleton preparation. This CLP (XP_809993) is considered a giant protein since the molecular weight is ≥519 kDa; however, the function of this protein remains unknown. To perform protein-protein interactions to find elements in *T. cruzi* cytoskeleton that interact with CLP, we generated a recombinant protein fragment (596 bp). This protein was cloned into pGEX-4T1 vector in fusion with the N-terminal Glutathione S-Transferase (GST) allowing generating a recombinant protein, named PG-GST (49 kDa). To obtain a soluble recombinant protein, we transformed it into two different *E. coli* strains (BL21(DE3)pLysS and Arctic Express, Agilent Technologies). Transformed bacterias were induced with 0,2 mM IPTG following specific conditions to express recombinant protein. We observed that only Arctic express strain was being able to produce a soluble fusion protein. Soluble PG-GST was used to immunize balb/c mice to produce specific polyclonal antibody to develop a molecular tool useful for immunoblotting, immunofluorescence, immunoprecipitation as well used to perform protein-protein interactions. The results will contribute to advance the studies of this giant protein in *T. cruzi*, which still has an unknown function and must play an essential role in the process of division and differentiation of the parasite. **Supported by:**PUB-USP **Keywords:** Giant protein; trypanosoma cruzi; cytoskeleton

PV66 - **FUNCTIONAL ANALYSIS OF DIVERGENT COMPONENTS FOR THE MRNA EXPORT IN TRYPANOSOMATIDS**

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The nucleocytoplasmic RNA export is an essential pathway for gene expression regulation in eukaryotic cells, but it is still poorly understood in protozoan parasites. Orthologs of only few proteins involved in mRNA export in higher eukaryotes are detectable. We previously described two conserved DEAD-box RNA helicases, components of the mRNA export pathway in T. cruzi: TcSub2, an essential for parasite survival and export of mRNA, and Hel45, a shuttling protein and component of mRNA complexes. Analyses of the interactomes of TcSub2 and Hel45 uncovered additional components of this system, including lineage-specific proteins of trypanosomatids, named as TcFOP-like, TcAPI5-like and TcNTF2-like. We confirmed that TcFOP-like and TcAPI5-like are nuclear proteins and colocalize with TcSub2, whereas TcNTF2-like is a cytoplasmic protein that co-localize with Hel45 and may migrates between nucleus and cytoplasm. We confirmed the interaction of TcFOP-like, TcAPI5-like and TcNTF2-like with mRNA export components and immunoprecipitation assays have been performed to identify new components by proteomic analysis. Knockdown of TcFOP-like, TcAPI5-like and TcNTF2-like shows that those proteins are not essential for the mRNA export but we still cannot exclude their role in this pathway. The knockdown of TcAPI5-like and TcFOP-like caused ultrastructural alterations such as condensation of the chromatin in the perinuclear region, mitochondrial disruption and multinucleated cell distribution. The mRNA export machinery in trypanosomes contains conserved and divergent proteins. Some proteins seem to be lineage-specific innovations and although they are not essential for parasite survival, their knockdown cause significative morphological effects. We suggest that these new components are likely part of the evolutionary adaptation to polycistronic transcription/trans-splicing. **Supported by:**CAPES, CNPq, FUNDAÇÃO ARAUCÁRIA, FIOCRUZ **Keywords:** Mrna export; trypanosomatids; tcsub2

PV67 - **INVESTIGATION AND CHARACTERIZATION OF RHABDOVIRAL SEQUENCES INSERTED IN THE GENOME OF SANDFLIES**

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In addition to Leishmania, sandflies are also vectors of some viruses. A considerable fraction of eukaryotic genomes is composed by exogenous genetic elements insertions of viral origin. Some of these insertions can confer advantages to the carrier, in a process called exaptation. One example of exaptation is the antiviral response.
In this work, we investigated the presence of endogenous rhabdoviral elements (ERE) in sandflies. These EREs are sequences coding for three different Nucleoproteins (NcP1; NcP2 and NcP3) and one RNA Polymerase RNA dependent (Pol) integrated into the genome of the *Lutzomyia longipalpis* embrionary cell lines LL-5 and Lulo and several sand flies species from Brazil, Argentina and Colombia. These viral elements are actively transcribed by these insects and cells. We are identifying flanking regions to determine insertions size by bioinformatics and molecular biology approaches.
Populations of sandflies studied showed differences regarding the number of EREs present in their genome, as well as which of these EREs were transcribed. *L. longipalpis* from Jacobina-BA carries and transcribes all three NcP EREs, while *L. longipalpis* from Puerto Iguazú-AR has two EREs, but transcribes only one.
In contrast, the Pol ERE was only found in LL-5 and Lulo cells, and in *L. longipalpis* from Lapinha-MG.
The presence of these viral fragments in some but not all sandflies raises important evolutionary questions and might be used as a tool for population genetics studies. When the insertion confers adaptive advantage the tendency is their maintenance in the population. If the insertion is neutral or a disadvante to the host, the tendency is their elimination from the population. The widespread presence of these EREs in different sand flies species from various geographical locations indicates an advantage for its presence. It will be also of great interest to investigate a functional role of these insertions on leishmania infection. **Supported by:**Fiocruz **Keywords:** Sandflies; insertion; rhabdovirus

PV68 - **TCBECLINA (VPS30/BECLIN1) OVEREXPRESSION IN *TRYPANOSOMA CRUZI* MAY AID PARASITE GROWTH UNDER NUTRITIONAL DEPRIVATION AND ACCELERATE THEIR DIFFERENTIATION**

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Autophagy is a eukaryotic conserved catabolic process in which cellular components are selectively degraded and recycled. Protein complexes such as class III phosphatidylinositol-3-kinase (Vps34-Beclin1-Vps15) are regulators in the process. Beclin1 protein (in mammals), a yeast Vps30/Atg6 orthologue, plays a pivotal role in regulating the Vps34 complex to induce autophagy as a response to diverse physiological signals. The autophagic process is essential for cellular survival and its dysfunction is related to different types of diseases. Given that *Trypanosoma cruzi* is an obligate intracellular eukaryotic parasite, it is possible that Vps34 complex is present in this parasite fulfilling a similar function. Thus, the aim of this study was to characterize the undescribed protein Vps30/Beclin1 for *T. cruzi* (Tcbeclina), and its interaction with other proteins, such as TcVps34 (already described for *T. cruzi*). The Tcbeclina hypothetic sequence has two conserved APG6 domains (different from yeasts and mammals which have only one APG6 domain) in the C-terminal region. The sequence was cloned into expression vectors (pTREX_mRFP and pROCK_Neo) in order to establish its possible location in the parasite, to perform its interaction with other proteins and to evaluate parasite survival and growth under nutritional deprivation. The constructs were transfected into epimastigotes of G strain and Cl-Brener clone and parasite clones were selected. Confocal immunofluorescence microscopy assays showed a possible interaction between Tcbeclina and TcVps34 pointing to a formation of a Vps34 autophagic complex as in other eukaryotes. Tcbeclina overexpression may play a role in processes such as survival, where it seemed to favor parasite growth under conditions of low nutrient concentration in the medium, and in parasite differentiation, in which occurred a faster differentiation of trypomastigote forms into extracellular amastigotes. **Supported by:**CNPq, FAPEMIG **Keywords:** Autophagy; trypanosoma cruzi; beclin1/vps30

PV69 - **DETECTION OF ACTIVE CHROMATIN REGIONS BY FAIRE-SEQ ANALYSIS IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES AND METACYCLICS FORMS**

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Gene expression in trypanosomes is remarkable as transcription is polycistronic, which means that RNA polymerase transcribes long transcripts containing several open reading frames, resulting in a lack of gene-specific regulation of early transcription. Moreover, these genes are organized in co-directional clusters with unrelated functions. It is believed that the promoter and transcription termination regions are in the intergenic regions between two clusters of divergent or convergent transcripts, respectively. These regions present several epigenetic alterations that can regulate the beginning and end of the transcription. In order to identify open chromatin regions present in *Trypanosoma cruzi* epimastigote (EPI) and trypomastigote metacyclic (META) life forms, FAIRE-seq assays were performed. GATK Best Practices pipeline was used to preprocess the reads and map them to *T. cruzi* Dm28c genome (accession PRJNA433042). Samtools was applied to remove alignment ambiguities using Q10 score. HOMER and MACS2 were used for peak calling. Peak annotation was carried out using UROPA and custom scripts. In general, peak sizes detected in EPI are larger than those present in META suggesting that the first has more active chromatin. In fact, it agrees with previous published data showing that META has more heterochromatin than EPI forms. Additionally, more tags were mapped to EPI life form, mainly transcription termination site features. Peaks found in META are mostly located at CDS, 5' and 3' UTR regions. Furthermore, some surface multigene families (DGF-1, GP63 and mucin) presented a higher number of peaks in META, while EPI revealed more peaks located at MASP and trans-sialidase genes. Visually, peaks were not distributed uniformly along the co-directional clusters in both EPI and META. We are currently evaluating the presence of differential active chromatin regions in different genomic features; including a parallel validation by FISH analysis. **Supported by:**FAPESP e CAPES **Keywords:** Epigenetics; faire-seq; trypanosoma cruzi

PV70 - **CHARACTERIZATION OF THE PROTEIN KINASE ATAXIA-TELANGIECTASIA MUTATED (ATM) OF LEISHMANIA MAJOR.**

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Double strands breaks (DSBs) are deleterious DNA lesions that trigger specific spatial and temporal patterns of the DNA damage response in eukaryotic cells. As part of the major routes to repair DSBs in eukaryotes, the activation of the protein kinase Ataxia-Telangiectasia Mutated (ATM) promotes the phosphorylation of many checkpoint-related factors such as p53, Chk2 and NBS1. The repair of DSBs in eukaryotes is carried out mainly through homologous recombination (HR), which operates in Leishmania cells, or non-homologous end joining (NHEJ), which seems to be absent in trypanosomatids. Since Leishmania parasites have an unusually plastic genome, characterized by hallmarks of genome instability such as, gene and chromosome copy number variation, mosaic aneuploidy and chromosome rearrangements, we set out to characterize the kinase ATM homolog of Leishmania major aiming at the investigation of its possible involvement in genome maintenance and stability. We used CRIPSR/cas9 genome editing to generate both, ATM null mutants (ATM-/-) and NmG3xmyc-ATM tagged cell lines. In vitro cultivation of ATM-/- promastigotes revealed a deficient growth pattern and a significant increase in the susceptibility to the genotoxic agent methyl methanosulfonate (MMS). We also observed that the formation and/or accumulation of phosphorylated histone H2A in response to DNA damage agents, was drastically affected in ATM-/- cells. Our current efforts are focused in the characterization of the patterns of ATM expression in Leishmania and its participation in genome maintenance and stability in cells exposed to distinct genomic injuries. **Supported by:**FAPESP **Keywords:** Dna damage response; genome stability; atm kinase

PV71 - ***TRYPANOSOMA CRUZI*** **RNA BINDING PROTEIN 42 CYTOPLASMIC FOCI FORMATION IS INDUCED BY GENOTOXIC STRESS**

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The parasite *Trypanosoma cruzi* is highly resistant to stress conditions. Although the mechanisms underlying this resistance remain unknown, RNA binding proteins (RBPs) may play essential roles in the stress response by accumulating into cytoplasmic granules. These structures could work as sorting centers for transcripts necessary for the parasite survival to stress. In this work, we investigate the localization of the protein RBP42 in the T. cruzi response to gamma radiation and benznidazole (BZ), both described as genotoxic stress. To investigate if TcRBP42 accumulates into granules under genotoxic stress, we transfected *T. cruzi* epimastigotes with a 6-his-tagged version of this protein (rTcRBP42), and performed immunofluorescence assays. GFP transfected parasites were used as control. We observed that under normal conditions, rTcRBP42 had a diffuse cytoplasmic distribution, but accumulates into cytoplasmic foci after 24 and 48 h of both gamma radiation (500 Gy) and BZ (120µM and 240µM). GFP remained diffusely distributed in the cytoplasm in all tested conditions. To investigate if the genotoxic treatments affected RNA localization, we performed immunofluorescence assay labeling newly-synthesized RNAs with a uridine analog. Under normal conditions, newly-synthesized RNAs were detected mainly in the nucleus and kinetoplast. After gamma radiation, newly-synthesized RNAs were diffusely distributed in the cytoplasm 24 h after irradiation, not being detected 48 h after irradiation. In contrast, BZ induced the aggregation of newly-synthesized RNAs into cytoplasmic foci after 24 and 48 h of treatment. Our results suggest that genotoxic stress conditions altered TcRBP42 localization into cytoplasmic foci after gamma radiation and BZ. Also, both gamma radiation and BZ altered newly-synthesized RNAs localization; however, only BZ caused RNA accumulation into granules, suggesting that *T. cruzi* stress response is dependent on the stress type. **Supported by:**CAPES, CNPq, FAPEMIG **Keywords:** Trypanosoma cruzi; rna binding protein; genotoxic stress

PV72 - **FUNCTIONAL CHARACTERIZATION OF ISWI CHROMATIN REMODELER IN *TRYPANOSOMA CRUZI***

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Chromatin remodeling is an essential mechanism to regulate DNA-dependent processes such as transcription, DNA repair, recombination and replication. ISWI (Imitation SWItch) is one of the nucleosome-remodeling multiprotein complexes that uses the energy of ATP hydrolysis to disrupt or alter the histone-DNA interaction. The main goal of this research is to characterize functionally the ISWI protein, a chromatin remodeler with unknown function in *T. cruzi*. For that, subcellular localization and interaction partners of this protein were investigated. The subcelullar localization of ISWI protein was assessed by indirect fluorescence microscopy using tagged ISWI protein at the N- or C- termini with GFP. In other organisms ISWI is an ATPase participating of chromatin remodelling complexes and as expected, the tagged protein localized to the nucleus of epimastigote forms. ISWI ATPase exists as part of complexes with a vast functional diversity which is determined by the associated proteins. To characterize ISWI protein interactions partners we applied an optimized workflow including cryogenic lysis, evaluation of different lysis buffer conditions, isolation of tagged complex using nanobody-conjugated magnetic beads and finally protein identification by mass spectrometry. Using this workflow, we identified previously known and news ISWI binding partners. Nucleoplasmin-like protein (NLP), regulator of chromosome condensation 1-like protein (RCCP) and phenylalanine/tyrosine-rich protein (FYRP) were the partners previously identified. These proteins were defined as the single major ISWI complex in *T. brucei* and were involved in the silencing of silent VSG expression sites and other transcriptionally inactive areas of the genome in *T. brucei*. After several attempts to knockout ISWI gene through CRISPR-Cas9 this was unsuccessful, suggesting an essential role of this gene. Additional assays are being performed to determine the regulatory function of this protein in this parasite. **Keywords:** Trypanosoma cruzi; iswi; interaction partners

PV73 - **BCY84_12113 GENE AS A NEW REGULATOR OF THE METACYCLOGENESIS PROCESS IN *TRYPANOSOMA CRUZI***

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Metacyclogenesis is one key process in the life cycle of the protozoa *Trypanosoma cruzi*. During this process, the parasite differentiates in the metacyclic trypomastigote stage, the infective form to the mammalian host. A few factors have been identified as triggers of this complex process and the information about the involved molecular events is scarce. In order to unraveling the mechanism involved in the metacyclogenesis of *T. cruzi*, we characterized a protein with unknown function coded by the BCY84_12113 gene of the Dm28c clone, which showed in a previous study modulated phosphorylation sites during this differentiation process. First, we determined the subcellular localization of the protein by indirect fluorescence microscopy using parasites expressing the tagged protein with GFP; the protein localized in the nucleus of epimastigote forms. Then, we used the CRISPR-Cas9 tool to obtain knockout parasites for this gene. Gene editing was confirmed and we evaluated the effect of the KO12113 in the metacyclogenesis process and infection. We found that the KO12113 parasites showed an increase of at least 3-fold in the number of metacyclic forms after inducing *in vitro* metacyclogenesis when compared with the wild-type parasite. These metacyclic forms were used to infect Vero cells at a ratio of 100:1 during 24 h and the number of infected cells were evaluated at 48, 72 e 96 h post-infection. We observed that KO12113 parasites showed a lower percentage of infected cells in all evaluation times relative to the wild-type parasites. Culture-derived trypomastigotes were observed after 5-days of infection in cells infected with the WT population, but these forms were not observed in cells infected with the KO12113 parasites in this period of time. Affinity purification assays are being performed to determine the interactions partners of this protein in an attempt to understand the participation of this protein in the metacyclogenesis process in *T. cruzi*. **Keywords:** Trypanosoma cruzi; metacyclogenesis; infection

PV74 - ***METARHIZIUM ANISOPLIAE* INFECTION REDUCES *TRYPANOSOMA CRUZI* LOAD IN THE VECTOR *RHODNIUS PROLIXUS***

SILVA, C.A.O.[*1]; ALVES, S.S.[1]; DE PAULA, A.R.[2]; SAMUELS, R.I.[2]; DA SILVA, J.L.N.[1]; MURY, F.B.[1]; DA SILVA, J.R.[1]
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Rhodnius prolixus is a vector of Chagas disease. The extensive use of pyrethroid insecticides to control triatomines has resulted in the development of resistant populations. The efficacy of the entomopathogenic fungus *Metarhizium anisopliae* was tested against *R. prolixus* during the nymphal stage and adults, proving the adults are more resistant to fungal infection, while nymphs are more susceptible. *M. anisopliae* infects insects through the cuticle and proliferates in the hemolymph, eliciting an immune response. The effect of fungal infection on parasite load has been studied in the malaria and *T. brucei* vectors. Here we investigated the effect of this fungus on the *T. cruzi* load in the midgut of adults *R. prolixus* and yet investigated the immune response of the vector during fungal infection. The experimental insects were allowed to feed on heparinized (2.5 units/ml) and heat-inactivated rabbit blood containing 10^7/ mL epimastigotes parasites through a latex artificial membrane. The engorded insects were infected with 5µL of a fungal suspension (10^7 conidia/mL) dropped under their wings. The control group was not infected with fungus. In order to analyze the expression of immune genes in the midgut and fat body, Dorsal and Cactus (toll), Relish (IMD), Stat (Jack-Stat) and Defensin were quantified by RT-qPCR 7 days after fungal infection. EF-1 was used as reference. Parasites were counted 7 and 17 days post-coinfection in a hemocytometer. After 7 and 17 days, the number of parasite was seriously reduced after coinfection. Analysis of immune genes in the coinfected insects showed that Stat and Defensin were upregulated in the fat body after 7 days, compared to the control. In the midgut, all the analyzed genes but Cactus (repressor of the Toll pathway) were upregulated 7 days after coinfection. The results showed that the *R. prolixus* is able to mount an immune response to fungal infection and the number of parasite is reduced by fungal infection. **Supported by:**FAPERJ **Keywords:** Immunity; biological control; chagas disease

PV75 - **ANALYSIS OF POST-TRANSLATIONAL MODIFICATIONS DIRECTED TO TWO EIF4G HOMOLOGUES IN LEISHMANIA INFANTUM.**

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In eukaryotes, translation initiation begins with the binding of the eIF4F complex (formed by eIF4A, eIF4E and eIF4G subunits) to the cap present in the 5' end of mRNAs, facilitating recognition by the ribosome. In trypanosomatids, five homologues of the eIF4G subunit are found, with two of them, EIF4G3 and EIF4G4, having similarities in structure and participating in two distinct eIF4F-like complexes. EIF4G3 plays a role in translation initiation while the function of EIF4G4 is unclear. This work aimed to identify post-translational modifications directed to EIF4G3 and EIF4G4 that might be required for their function/regulation. Leishmania infantum promastigotes were thus transfected with plasmidial constructs that allow expression of HA-tagged versions of the two proteins. Western blot analyzes showed that each of the tagged proteins were represented by two isoforms, in an expression profile similar to the endogenous EIF4G3 and EIF4G4 proteins. Growth curves of the transfected promastigotes did not show differences when compared to wild type cells and the overall abundance of the ectopic proteins was essentially constant. For EIF4G3, its two isoforms were found in all stages of the growth curve. In contrast, for EIF4G4, the higher isoform was expressed only late in the growth curve, after 72 hours of culture, indicating a specific pattern of post-translation modification in stationary phase cells. Next, selected mutants were also generated having substitutions in amino acid residues implicated in binding to eIF4E and eIF4A homologues. When the mutant HA-tagged proteins were similarly assayed, the EIF4G3 mutant LNK67-69AAA, impaired in eIF4A binding, was detected in much reduced levels and represented by a single isoform. The presence of isoforms for both proteins is suggestive of regulation by phosphorylation, which, for EIF4G3, might require binding to its EIF4AI partner. Tentative phosphorylation motifs were identified that need to be experimentally confirmed. **Supported by: Keywords:** Leishmania; phosphorylation; protein synthesis

PV76 - **SPATIAL AND TEMPORAL ANALYSIS OF MORBIDITY AND MORTALITY BY VISCERAL LEISHMANIASIS IN AN ENDEMIC AREA OF NORTHEASTERN BRAZIL**

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Introduction: Visceral leishmaniasis (VL) is a neglected and tropical disease potentially fatal if not treated in a timely manner. It has been presenting important changes in its epidemiological dynamics. Brazil concentrates 96% of VL cases in the Americas and the Northeast region accounted for 56% of VL deaths in the country between 2000-2011. Objective: To analyze the spatiotemporal distribution patterns of VL-related morbidity and mortality in Sergipe from 2000-2017. Method: We carried out an ecological and time series study. All cases and deaths due to VL recorded in the period 2000-2017 were included. The LV incidence, mortality and lethality coefficients were calculated using data obtained from the Notification Disease Information System (Sinan). The risk areas were stratified by calculating the three-year composite LV indicator (ICLV), as recommended by the Pan American Health Organization (PAHO) in April 2019. Results: During the 18-year period, 971 cases of VL (53.9±18.8 cases/year), 38 cases of co-infection LV-HIV (2.1 cases/year) and 106 deaths (5.9±3.2 deaths/year) related to VL directly or indirectly in more than one third of the state (11 to 29 municipalities/year) were reported. There was a higher prevalence of cases among children and adolescents (55.4%), with non-white skin color (68.6%), male (65.7%), urban/periurban (64.5%), with low education (40.8%) and the cure rate was 82.5%. State incidence rates and case fatality ranged from 1.01-5.92/100.000 inhabitants to 2.86-25.49%, respectively. The spatial stratification of the VL risk transmission presented dynamic over time, however, the metropolitan area remained high transmission intense in the last three-year periods, especially in the capital. Conclusion: These study results demonstrate an urbanization of the disease and, despite the measures instituted by the VL Surveillance and Control Program, its incidence and lethality do not present significant reductions. **Supported by:**CNPq/FAPITEC/SE/SES **Keywords:** Spatial analysis; epidemiology; visceral leishmaniasis

PV77 - **POLYOMICS APPROACHES AND TARGET VALIDATION OF THE MOA AND RESISTANCE OF A NOVEL ANTILEISHMANIAL IN *LEISHMANIA SPP***

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Leishmaniasis is a Neglected Tropical Disease (NTD) caused by many species of the parasite Leishmania. Sphingolipids and sterols are specific components of the membrane in Leishmania that are different than their mammalian counterpart. Sphingolipids and sterols are associated in the membrane of the parasite forming the lipids rafts, and due to their selectivity, these two molecules are ideal drug candidates. The sphingolipid inositol phosphorylceramide synthase (IPCS), and ergosterol are antileishmanial target (Denny et al, 2006, Pountain et al. 2019). Four compounds were selected from a library of >1k, the most potent (CL) was active against promastigotes and amastigotes of several Leishmania spp., CL also inhibited the L. major IPC synthase. Using untargeted metabolomics and lipidomics, we investigated the mechanism of action (MoA) of CL which is related with sphingolipids and the metabolism of sterols (unpublished), combined with Next Generation Sequencing (NGS) we intend to facilitate the deconvolution of the MoA of CL in various Leishmania species selected for resistance against CL. Resistant mutants growth rate was stable at concentrations of CL between 6.2 to 14-fold, while EC50 values increased by 2 to 2.5-fold, with respect to their parental wild type lines cultured in parallel without drug. Importantly, no cross resistance has been observed against any of the antileishmanials tested (miltefosine, amphotericin B, pentamidine, antimonials and paromomycin). Further characterisation of these mutants will be carried out using several mammalian cell lines and *in vivo* assays. **Supported by: Keywords:** Antileishmanials; drug resistance; target validation